*Forest Sci.*, Vol. 28, No. 3, 1982, pp. 423–458
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# *Patterns of Ectomycorrhizal Host Specificity and Potential Among Pacific Northwest Conifers and Fungi*

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ABSTRACT. Twenty-seven fungus species with diverse sporocarp-host associations were tested for ectomycorrhiza formation with seven Pacific Northwest conifers: *Pseudotsuga menziesii*, *Tsuga heterophylla*, *Larix occidentalis*, *Picea sitchensis*, *Pinus contorta*, *P. ponderosa*, and *P. monticola*. The fungi varied widely in their ability to form mycorrhizae with the various conifers but can be placed into three groups: (1) fungi with wide ectomycorrhizal host potential, low specificity, sporocarps usually associated with diverse hosts in the field, (2) fungi with intermediate host potential yet specific or limited in sporocarp-host associations, and (3) fungi with narrow host potential, only able to form ectomycorrhizae with a specific host species or species within a genus and likewise limited in their sporocarp association. Numerous fungi which produce sporocarps exclusively in association with specific hosts were able to form well-developed ectomycorrhizae with one or more nonassociated hosts. Thus, specific sporocarp-host association does not necessarily limit mycorrhizal associations with other hosts. Often, however, fungi with known sporocarp-specific hosts showed best mycorrhiza development with that particular host suggesting further specialization in those associations. Among the conifers, the three *Pinus* spp. showed little infrageneric differences in their ability to form ectomycorrhizae with the various fungi. *Pseudotsuga menziesii* and *Larix occidentalis* were able to form ectomycorrhizae with fungi that fruit only with one or the other.

The fungi with wide sporocarp-host associations showed no incompatibility with any of the hosts, and it is suggested that they may share a compatibility or recognition factor common to many ectomycorrhizal hosts. The specialized fungi with specific sporocarp-host associations often showed incompatibility with other nonassociated hosts. Disruption of the cortex by the invading fungus and lignification as indicated by intense safranin staining of cortical cells were the most common indicators of incompatibility. This host reaction suggests a type of phenolic defense mechanism as displayed in many plant-pathogen interactions. The need for future research on the mechanisms and factors determining ectomycorrhizal host-fungus compatibility and specificity is emphasized. FOREST SCI. 28:423–458.

ADDITIONAL KEY WORDS. Symbionts, mycorrhizae, mycology.

ALTHOUGH MYCOLOGISTS have long recognized ectomycorrhizal fungi to differ in host associations, detailed information on the specific nature of these associations is lacking. Many fungi, such as *Amanita muscaria* (L. ex Fr.) Pers. ex Hook., *Boletus edulis* Bull. ex Fr., and *Laccaria laccata* (Scop. ex Fr.) Bk. and Br. commonly associate with diverse hosts (Trappe 1962) and have been termed

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"broad host-ranging fungi." Others, such as many *Suillus* and *Rhizopogon* species, fruit only in association with a particular host species or species within a genus and have been called "host-specific fungi." For the most part, such recognition has come from repeated field observations of sporocarp-host associations. By the pure culture synthesis method of Melin (1921), many of these associations have been proven to be ectomycorrhizal. Yet, much remains to be learned about the various types and degrees of specificity involved in ectomycorrhizal symbioses.

Few studies have addressed the ectomycorrhizal host potential, i.e., the ability to form ectomycorrhizae, that mycelium of "host-specific" fungi may have with other "nonassociated" hosts. Trappe (1962) suggested that although sporocarps may only form with a particular host or species within a genus, mycorrhiza formation with other hosts should not be ruled out. This was later demonstrated by Grand (1968) and Linnemann (1971) who synthesized ectomycorrhizae between the well-known *Larix*-specific fungus *Suillus grevillei* (Klotzsch) Sing. and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Similarly, Suggs and Grand (1972) found *Suillus* spp. that normally fruit only with five-needled pines also form ectomycorrhizae with two- and three-needled pines. Molina (1979, 1980b, 1981) and Molina and Trappe (1982) have provided further evidence to support this phenomenon. Thus, the ectomycorrhizal host potential of fungal symbionts may differ strongly from that observed simply from sporocarp-host associations.

Host symbionts also vary in their ability to form ectomycorrhizae among diverse fungi. Molina and Trappe (1982) suggest that a spectrum exists from unspecialized, highly receptive hosts to more specialized and restrictive hosts. For example, the ericaceous hosts *Arbutus menziesii* Pursh and *Arctostaphylos uva-ursi* (L.) Spreng. are broadly receptive to numerous ectomycorrhizal fungi, including many which are strongly sporocarp-specific with other hosts (Molina and Trappe 1982). At the opposite extreme, *Alnus* species appear highly restrictive towards their fungal associates, including many fungi with broad host ranges (Molina 1979, 1981). Such comparative information for other ectomycorrhizal hosts is highly desirable.

Fungus-host specificity or lack thereof also implies differences in compatibilities between the two symbionts. Yet little information is available on the physiochemical processes which determine ectomycorrhizal specificity and compatibility. Molina (1981) found *Alnus* roots reacted strongly to colonization by the ectomycorrhizal fungus *Paxillus involutus* (Batsch ex Fr.) Fr.; cortical cells adjacent to mantle mycelium became lignified, as indicated by safranin staining, forming a barrier which the fungus rarely penetrated. Malajczuk and others (1982) and Molina (1980b) have found similar phenolic responses in other incompatible host-fungus interactions. Such preliminary data is essential to pursuing more detailed research on the recognition factors and mechanisms determining ectomycorrhizal specificity and compatibility. It may well be that processes similar to those operating in the specificity of and resistance to plant diseases may also be involved in ectomycorrhizal specificity.

To gain further insight into the complexities of ectomycorrhizal host potential and specificity, a large scale pure culture synthesis study between diverse Pacific Northwestern fungi and conifers was conducted. The specific objectives were to (1) determine the degree to which ectomycorrhizal fungi of known specific sporocarp-host association can form ectomycorrhizae with nonassociated hosts, (2) compare the ectomycorrhizal host potentials of fungi with broad host ranges, (3) compare patterns of ectomycorrhiza formation and specificity of different conifer hosts inoculated with diverse fungi, and (4) establish macro- and microscopic indicators of ectomycorrhizal compatibilities and incompatibilities among the various host-fungus confrontations.

## MATERIALS AND METHODS

Table 1 lists the 27 fungal isolates selected for this study along with their associated hosts and isolation background. The specimens from which isolates were obtained are deposited in the Oregon State University Herbarium. Also included are data of other host associations from our past collections. The fungi were selected to include species both of well-known host-specific and non-host-specific associations as well as many with little or no previous experimental evidence of ectomycorrhizal association. Fungus collections were made primarily in the fall of 1976 in Oregon and Washington. Source habitats varied from coastal sand dunes to interior mountain ranges and valleys. All fungi were isolated from sporocarp tissue except *Cenococcum geophilum* Fr., which was isolated from a surface sterilized sclerotium (Trappe 1969). Isolations were made in the field immediately after collection or as soon as possible upon returning to the laboratory.

Seven Pacific Northwestern conifer hosts were selected for this study: Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western larch (*Larix occidentalis* Nutt.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), lodgepole pine (*Pinus contorta* Dougl.), ponderosa pine (*Pinus ponderosa* Dougl.), and western white pine (*Pinus monticola* Dougl.). The three *Pinus* species were selected to detect possible infrageneric differences in ectomycorrhiza formation. Originally *Tsuga mertensiana* (Bong.) Carr. and *Abies procera* Rehd. were to be included in this study, but we could not surface sterilize the seeds.

For rearing aseptic seedlings, seeds were first cleansed by agitating in cool running tapwater (1 h), followed by shaking in a jar containing two to three drops of the surfactant Tween 20 (1 h), and finally rinsed in cool running tapwater (1 h). Seeds were then surface sterilized by soaking for 1 h in 30 percent $H_2O_2$, except for western hemlock which only received a 30-min soaking. After a rinse with 2 liters sterile distilled water, seeds were aseptically planted into small screw-topped vials half-filled with nutrient agar. Seeded vials were observed for 1 week and all contaminated vials were discarded. Remaining vials were placed into refrigeration (3°C) for seed stratification. After 30 days, vials with seeds were placed under fluorescent-incandescent light (ca. 10,500 lx) to germinate.

The pure culture synthesis design of Molina (1979) was used for all fungus-host combinations. Large glass test tubes, 300- × 38-mm, partially filled with a 1:12 peat moss-vermiculite substrate saturated with modified Melin-Norkrans nutrient solution (MMN) (Marx 1969) and autoclaved for 15 min at 121°C, were used as the synthesis vessels. Fungus inoculum was prepared by growing isolates for 3–4 weeks at 20°C in screw-capped glass culture tubes filled with 15 ml MMN nutrient solution and small chips of broken glass; cultures were shaken periodically to fragment the growing mycelium. Germinants were aseptically planted into the sterile synthesis tubes and inoculated with the previously prepared fungus culture in an air-filtered transfer room. Synthesis tubes were then placed under fluorescent-incandescent light of ca. 10,500 lx set for a 15-h photoperiod with the bottom half of the tubes submerged in a water bath of ca. 16°C for the duration of the experiment.

After 6 months, the seedlings were removed intact from the synthesis tubes and their roots gently washed free of substrate. For each inoculated seedling, the degree of ectomycorrhiza development was determined by visually ranking the approximate percentage of total short roots colonized into five groupings: less than 5 percent, 5–25 percent, 26–50 percent, 51–75 percent, and 76–100 percent (see Table 2 footnote). Roots were examined with the aid of a stereomicroscope for external ectomycorrhiza characteristics, and cross sections of colonized roots were examined for Hartig net development. In addition to free hand sections,

*TABLE 1. Isolation year, source, and associated hosts of ectomycorrhizal fungi tested in pure culture syntheses with seven Pacific Northwest conifers.*

| Fungus species and isolate no.[1] | Year of isolation | Source location and elevation | Associated hosts[2] | Other hosts[3] |
|---|---|---|---|---|
| *Alpova diplophloeus*, S-276 | 1976 | Cowlitz Co., WA. 600 m | *Alru-Psme* | *Alnus* spp. |
| *Amanita muscaria*, S-230 | 1976 | Benton Co., OR. 1,200 m | *Abpr* | *Psme, Pisi, Tsme, Tshe, Pico, Pimo, Abies* spp., *Potr* |
| *Astraeus pteridis*, S-237 | 1976 | Jackson Co., OR. 1,020 m | *Psme* | *Psme, Tshe* |
| *Boletus edulis*, S-329 | 1976 | Tillamook Co., OR. 20 m | *Pisi* | *Pisi, Tsme, Tshe, Abies* spp., *Psme, Pipo, Pico, Quercus* spp. |
| *Cenococcum geophilum*, A-145 | 1974 | Lane Co., OR. 700 m | *Psme* | (see Trappe 1964) |
| *Cortinarius pistorius*, S-301 | 1976 | Union Co., OR. 1,410 m | *Pipo-Psme* | — |
| *Fuscoboletinus aeruginascens*, S-281 | 1976 | Union Co., OR. 1,410 m | *Laoc* | *Laoc.* |
| *Gastroboletus subalpinus*, S-395 | 1977 | Hood River Co., OR. 2,000 m | *Pial* | *Pial, Tsme, Tshe* |
| *Hysterangium separabile*, S-277 | 1976 | Benton Co., OR. 750 m | *Psme-Tshe* | *Psme, Tshe, Pipo* |
| *Laccaria laccata*, S-238 | 1976 | Klamath Co., OR. 1,500 m | *Tsme* | *Psme, Tsme, Abies* spp., *Pisi, Pico, Pipo* |
| *Lactarius deliciosus*, S-229 | 1976 | Jackson Co., OR. 1,020 m | *Pipo-Psme* | *Pisi, Abies* spp., *Pico, Psme, Tshe* |
| *Leccinum manzanitae*, S-346 | 1976 | Tillamook Co., OR. 20 m | *Arco-Aruv-Pico* | *Arme-Psme* |
| *Melanogaster intermedius*, S-385 | 1977 | Linn Co., OR. 45 m | *Psme-Quga* | — |
| *Paxillus involutus*, S-403 | 1977 | Linn Co., OR. 45 m | *Coav* | *Bepe, Potr, Tshe, Pico* |
| *Pisolithus tinctorius*, S-216 | 1976 | Coos Co., OR. 900 m | *Psme* | *Abies, Pipo, Quercus, Bepe* |
| *Rhizopogon cokeri*, S-215 | 1976 | Jackson Co., OR. 1,140 m | *Pipo-Pico-Psme* | *Tsme, Abies* |
| *Rhizopogon fuscorubens*, S-294 | 1976 | Lane Co., OR. 5 m | *Pico* | *Pico-Pisi* |
| *Rhizopogon occidentalis*, S-297 | 1976 | Tillamook Co., OR. 20 m | *Pico* | *Pipo, Abies* |
| *Rhizopogon vinicolor*, A-153 | 1975 | Benton Co., OR. 450 m | *Psme-Tshe* | *Psme, Tshe* |
| *Scleroderma hypogaeum*, S-315 | 1976 | Tillamook Co., OR. 30 m | *Pico-Psme-Aruv* | — |
| *Suillus brevipes*, S-223 | 1976 | Jackson Co., OR. 1,080 m | *Pico-Psme* | *Pico, Abies* spp., *Tsme* |

*TABLE 1. Continued.*

| Fungus species and isolate no.[1] | Year of isolation | Source location and elevation | Associated hosts[2] | Other hosts[3] |
|---|---|---|---|---|
| *Suillus cavipes*, S-298 | 1976 | Union Co., OR. 1,440 m | *Laoc* | *Laoc-Psme-Abies* spp. |
| *Suillus grevillei*, S-255 | 1976 | Union Co., OR. 1,440 m | *Laoc* | — |
| *Suillus lakei*, S-243 | 1976 | Benton Co., OR. 450 m | *Psme* | *Psme-Tsme-Abies* spp. |
| *Tricholoma flavovirens*, S-344 | 1976 | Tillamook Co., OR. 20 m | *Pico-Aruv* | *Pico, Pipo* |
| *Truncocolumella citrina*, S-273 | 1976 | Jackson Co., OR. 1,020 m | *Psme* | *Psme, Abies* |
| *Zelleromyces gilkeyae*, S-306 | 1976 | Cowlitz Co., WA. 600 m | *Psme-Alru* | *Psme, Tshe* |

[1] Herbarium numbers of voucher collections are listed in Molina (1979) except: *G. subalpinus* Trappe 5058, *M. intermedius* Fogel 1578.

[2] Ectomycorrhizal hosts in immediate vicinity of sporocarp collection. Abbreviations: *Abpr, Abies procera*; *Alru, Alnus rubra*; *Arco, Arctostaphylos columbiana*; *Arme, Arbutus menziesii*; *Aruv, Arctostaphylos uva-ursi*; *Bepe, Betula pendula*; *Coav, Corylus avellana*; *Laoc, Larix occidentalis*; *Pial, Pinus albicaulis*; *Pico, Pinus contorta*; *Pimo, Pinus monticola; Pipo, Pinus ponderosa*; *Pisi, Picea sitchensis*; *Potr, Populus trichocarpa*; *Psme, Pseudotsuga menziesii*; *Quga, Quercus garryana*; *Tshe, Tsuga heterophylla*; *Tsme, Tsuga mertensiana*.

[3] From unpublished collection data of J. M. Trappe, 1965–1980.

colonized roots representative of each fungus, especially those with unusual or questionable ectomycorrhizal features, were embedded in paraffin, microtome-sectioned to 12-$\mu$m thickness, and stained with safranin-fast green (Johansen 1940). Data were taken on the degree of ectomycorrhizal colonization, external characters (size, texture, morphology), mantle and Hartig net development, staining reactions, and the overall appearance of the root cortex. Regular development of a Hartig net was the final criterion on which the ectomycorrhizal status of colonized roots was determined.

Fungus-host combinations in which root-fungus contact was poor and ectomycorrhizae did not form during the initial experiment were repeated. The repeat inoculations were carefully observed and grown until continued contact between roots and fungus insured that ectomycorrhizae could form. Negative results were then judged to indicate incompatibility between host and fungus.

## RESULTS AND DISCUSSION

All hosts grew well in the synthesis tubes, reaching average heights after 6 months as follows: Douglas-fir, 8.5 cm; western larch, 8.0 cm; Sitka spruce, 7.5 cm; ponderosa pine, 7.0 cm; western hemlock, 6.5 cm; western white pine, 6.0 cm; lodgepole pine, 5.5 cm. Root systems were well developed. Most fungi grew well in the synthesis tubes and had completely penetrated the peat-vermiculite substrate within 3 to 4 months. *Boletus edulis, Hysterangium separabile* Zeller, *Leccinum manzanitae* Thiers, and *Zelleromyces gilkeyae* Sing. and Smith grew more slowly than the other fungi and were often restricted to dense mycelial patches rather than uniform penetration of the entire substrate.

*TABLE 2. Degree of ectomycorrhiza formation between 27 fungi and 7 Pacific Northwestern conifers in pure culture syntheses.*[1]

| Fungus species | Conifer hosts[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Psme* | *Laoc* | *Tshe* | *Pisi* | *Pimo* | *Pipo* | *Pico* |
| *Alpova diplophloeus* | –0– | –0– | –0– | –0– | –0– | –0– | –0– |
| *Amanita muscaria* | ++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| *Astraeus pteridis* | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| *Boletus edulis* | NC | ++ | + | + | +++ | +++ | ++++ |
| *Cenococcum geophilum* | ++++ | + | ++++ | +++ | +++ | +++ | +++ |
| *Cortinarius pistorius* | –0– | –0– | –0– | –0– | + | ++ | +++ |
| *Fuscoboletinus aeruginascens* | ++++ | ++++ | –0– | ++ | –0– | –0– | –0– |
| *Gastroboletus subalpinus* | T | –0– | –0– | –0– | ++ | + | +++ |
| *Hysterangium separabile* | NC | + | + | NC | + | –0– | ++ |
| *Laccaria laccata* | ++++ | ++++ | ++++ | ++++ | +++ | ++++ | ++++ |
| *Lactarius deliciosus* | +++ | ++ | ++++ | +++ | +++ | ++++ | ++++ |
| *Leccinum manzanitae* | + | –0– | ++ | + | NC | + | + |
| *Melanogaster intermedius* | ++++ | ++++ | ++++ | ++++ | +++ | ++++ | ++++ |
| *Paxillus involutus* | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| *Pisolithus tinctorius* | ++++ | ++++ | +++ | ++++ | ++++ | ++++ | ++++ |
| *Rhizopogon cokeri* | –0– | –0– | –0– | –0– | +++ | ++ | ++ |
| *Rhizopogon fuscorubens* | –0– | + (?) | –0– | ++ (?) | NC | +++ | ++++ |
| *Rhizopogon occidentalis* | –0– | –0– | –0– | T (?) | + | NC | ++++ |
| *Rhizopogon vinicolor* | ++++ | +++ | T | ++ (?) | T (?) | –0– | –0– |
| *Scleroderma hypogaeum* | +++ | ++ | +++ | +++ | +++ | ++ | ++++ |
| *Suillus brevipes* | ++++ | +++ | –0– | ++ (?) | ++++ | +++ | ++++ |
| *Suillus cavipes* | +++ | +++ | T (?) | –0– | ++ | –0– | + |
| *Suillus grevillei* | ++++ | ++++ | –0– | –0– | NC | T (?) | ++ (?) |
| *Suillus lakei* | ++++ | ++ | +++ | +++ | +++ | + (?) | ++++ |
| *Tricholoma flavovirens* | ++ | ++ | +++ | ++ | ++ | ++ | +++ |
| *Truncocolumella citrina* | +++ | ++++ | –0– | –0– | –0– | –0– | –0– |
| *Zelleromyces gilkeyae* | + | –0– | + | –0– | –0– | –0– | –0– |

[1] Approximate percentage groupings of ectomycorrhizae to total short roots: –0– = No ectomycorrhiza formation; T = Trace, Less than 5 percent; + = 5–25 percent; ++ = 26–50 percent; +++ = 51–75 percent; ++++ = greater than 75 percent; NC = Synthesis not completed. Those groupings followed by (?) indicate colonized roots with unusual or questionable colonizations; readers are referred to the text for explanations of these characters.

[2] See Table 1 for host abbreviations.

Table 2 presents the results of the pure culture synthesis attempts and the degree of ectomycorrhiza formation. The degree of ectomycorrhiza formation varied greatly among the numerous host-fungus combinations. For individual fungi, however, ectomycorrhiza formation varied less between host species. In most cases, it was directly related to the growth rate of the fungus. Although slow growing isolates colonized fewer feeder roots, the ectomycorrhizae formed were well developed.

All fungi except *Alpova diplophloeus* (Zeller and Dodge) Trappe and Smith formed ectomycorrhizae and with more than one host. Color and texture characteristics for individual fungi remained nearly constant regardless of the host. Ectomycorrhiza morphology was host dependent; colonized *Pinus* feeder roots developed into typical bifurcate structures whereas mycorrhizae of remaining hosts matured into pinnate and variably branched forms.

Overall, patterns of ectomycorrhiza formation and specificity varied considerably among the fungi. A number of host-specific fungi were able to form ec-

tomycorrhizae with other hosts. Many host-fungus combinations also yielded colonized roots of abnormal or questionable ectomycorrhizal status, indicating differences in compatibility between the symbionts. Details of ectomycorrhiza formation are described below by individual fungus, followed by discussion of comparisons of ectomycorrhizal host potential, specificity, and compatibility among fungi and hosts.

## Synthesis Descriptions

### *Alpova diplophloeus* (Zeller and Dodge) Trappe and Smith

*A. diplophloeus* failed to form ectomycorrhizae with any of the conifers, although many hosts superficially appeared to have formed abundant ectomycorrhizae. Feeder roots were generally much branched and well mantled with a loose-lying to crustlike mycelial mat. *Pinus* spp. short roots branched dichotomously, often repeatedly so, while Douglas-fir and western larch short roots branched variably. Mantled roots also became dark brown to near black with age.

Cross sections revealed the mantles to be mostly fragmentary. No regularly developed Hartig net was seen. Only occasionally did the fungus penetrate the root and then appeared disruptive to the tissue. Overall, mantled feeder roots appeared unhealthy and reacted strongly to the presence of the fungus. Numerous cortical cells, especially the outer cells in direct contact with the surface mycelium, were in various stages of collapse and often deeply safranin stained (Fig. 1). Safranin selectively stains lignified tissue (Johansen 1940).

*A. diplophloeus* invariably fruits beneath *Alnus* spp. in the Pacific Northwest (Trappe 1975) and was recently found beneath *Alnus glutinosa* (L.) Gaertn. in Europe (Clémençon 1977). In previous pure culture syntheses, Molina (1979, 1981) found *A. diplophloeus* able to form well-developed ectomycorrhizae with five *Alnus* spp. In these syntheses, mycorrhizae were consistently pale brown to brown, with thick, tightly interwoven mantles and Hartig nets which uniformly penetrated the cortex; cortical cells did not become stained with safranin. In addition to the inability to form ectomycorrhizae with the tested conifer hosts, Molina and Trappe (1982) found *A. diplophloeus* unable to form arbutoid mycorrhizae with the nonrestrictive hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi*. Thus, *A. diplophloeus* appears highly specialized and specific to *Alnus* both in sporophore association and ectomycorrhiza formation.

### *Amanita muscaria* (L. ex Fr.) Pers. ex Hook.

*A. muscaria* formed well-developed and abundant ectomycorrhizae with all hosts. Ectomycorrhizae were bright white with a finely tomentose to smooth-appressed mantle surface; well-swollen elements contrasted sharply with uncolonized roots (Figs. 9 and 10). Thick white hyphal strands emanated from the ectomycorrhizae and through the peat-vermiculite substrate. Prolific branching and elongation of the ectomycorrhizae occurred on all hosts. *Pinus* spp. ectomycorrhizae varied from simple to compoundly bifurcate, 1–7 mm long, often densely clustered along lateral roots. Those of all other hosts varied from simple to pinnate or variably branched, 1–20 mm long; western larch ectomycorrhizae were tortuous (Fig. 10).

Serial cross sections showed classical ectomycorrhizal anatomy. Mantles composed of tightly interwoven fine hyphae were mostly uniform in thickness and often very thick, up to 100 $\mu$m on western hemlock and 115 $\mu$m on western larch (Fig. 2). Hartig nets uniformly penetrated two to four cortical cell layers. A well-developed tannin layer was noted on Sitka spruce ectomycorrhizae.

*A. muscaria* is well known for its wide host range, so it was not surprising that it formed ectomycorrhizae with all conifers tested. It has been reported to as-

sociate with 12 genera of ectomycorrhizal hosts (Trappe 1962, Malajczuk and others 1982, and Molina and Trappe 1982) including successful pure culture syntheses with seven *Pinus* spp. (Trappe 1962, Riffle 1973), *Larix decidua* Mill. (Melin 1925b), *Picea abies* (L.) Karst (Melin 1925a; Modess 1939, 1941), *Pseudotsuga menziesii* (Linnemann 1969), *Betula pendula* Roth (Melin 1923, Mason 1974), several *Eucalyptus* species (Malajczuk and others 1982), *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982).

*Astraeus pteridis* (Shear) Zeller

*A. pteridis* formed well-developed ectomycorrhizae with all hosts similar to those described by Trappe (1967) on Douglas-fir, often colonizing entire short root systems. Ectomycorrhizae were initially pale brown, then darkened to a golden brown occasionally with a slight metallic silvery sheen. Mantle surfaces were tightly appressed to smooth, with abundant dark brown emergent hyphal strands. Numerous dichotomously branching coralloid clusters, up to 14 mm across, formed on lodgepole and ponderosa pines. On western white pine, however, ectomycorrhizae were mostly elongate cylindric to pinnate fans (Fig. 11), up to 15 mm long, unusual for *Pinus* ectomycorrhizae. Douglas-fir, western hemlock, and western larch ectomycorrhizae were sometimes simple but mostly variably branching or occasionally pinnate, 2–10(–15) mm long. Sitka spruce ectomycorrhizae were mostly simple, occasionally branching, 3–12 mm long.

Cross sections revealed tightly interwoven mantles with appressed surfaces, ranging from 12–25 $\mu$m thick on western larch to 40–60 $\mu$m on western white pine (Figs. 3 and 4). Hartig nets uniformly penetrated two to three cortical cell layers. A tannin layer was noted in ponderosa pine ectomycorrhizae.

We have only occasionally collected *A. pteridis* in the Pacific Northwest, always associated with Douglas-fir except for one collection in a pure ponderosa pine stand. Smith (1975) lists its major habitat as stands of Douglas-fir "but not limited to a single tree species." Trappe (1967) established an ectomycorrhizal association of *A. pteridis* with Douglas-fir by pure culture synthesis. In other pure culture syntheses, *A. pteridis* has formed ectomycorrhizae with five *Alnus* spp. (Molina 1979, 1981), several *Eucalyptus* spp., and *Pinus radiata* D. Don (Malajczuk and others 1982); and arbutoid mycorrhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982). From the results of this study and those cited previously, *A. pteridis* has a wide potential for mycorrhiza formation with numerous hosts. This, along with its prolific mycorrhizae formation, makes it a prime candidate for inoculation of forest nurseries.

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FIGURES 1–8. Cross sections of synthesized ectomycorrhizae and colonized feeder roots. All figures ×170 (m, mantle; H, Hartig net; S, safranin stained cells).

FIG. 1. *Pinus ponderosa* feeder root colonized by *Alpova diplophloeus*. Note the collapsed outer cortical cells, stained deeply by safranin.

FIG. 2. *Amanita muscaria* + *Larix occidentalis* ectomycorrhiza.

FIG. 3. *Astraeus pteridis* + *Tsuga heterophylla* ectomycorrhiza.

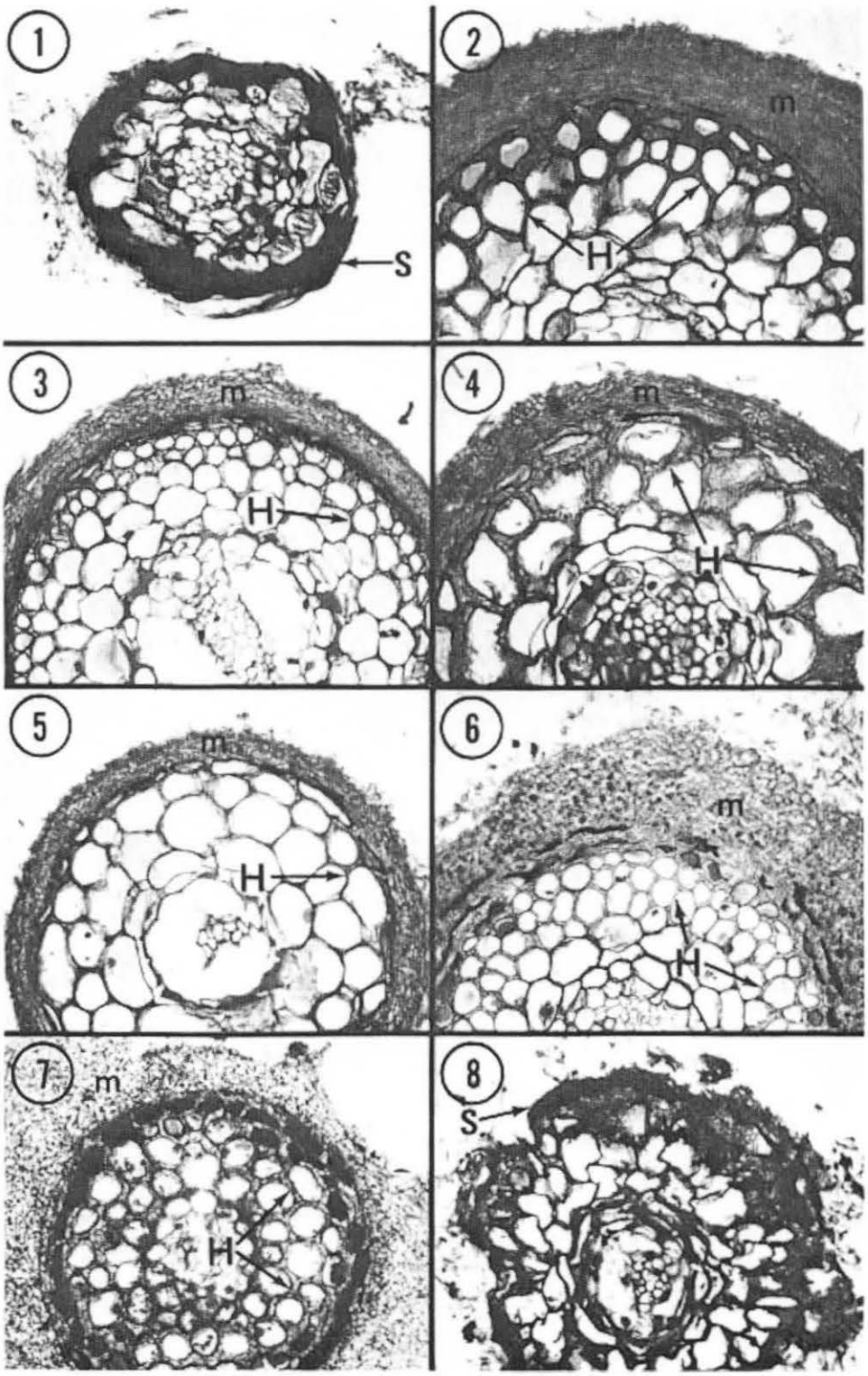
FIG. 4. *Astraeus pteridis* + *Pinus monticola* ectomycorrhiza.

FIG. 5. *Boletus edulis* + *Pinus contorta* ectomycorrhiza.

FIG. 6. *Fuscoboletinus aeruginascens* + *Larix occidentalis* ectomycorrhiza.

FIG. 7. *Fuscoboletinus aeruginascens* + *Picea sitchensis* ectomycorrhiza.

FIG. 8. *Pinus contorta* feeder root colonized by *Fuscoboletinus aeruginascens*. Note the disruption of the cortex and safranin staining of outer cortical cells.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*Boletus edulis* Bull. ex Fr.

*B. edulis* formed ectomycorrhizae with all hosts tested. Although ectomycorrhizae were most numerous on the three *Pinus* spp., the few ectomycorrhizae formed with western hemlock, western larch, and Sitka spruce were equally well developed. Ectomycorrhizae were white to tan with a finely pubescent to smooth-appressed mantle surface and swollen, almost succulent, in sharp contrast to uncolonized roots. *Pinus* ectomycorrhizae were simple to compoundly bifurcate, 1–7 mm long. Western hemlock and Sitka spruce ectomycorrhizae remained simple, 2–4 mm long, while those of western larch became variably branched, up to 8 mm long, and noticeably tortuous (Fig. 12).

Cross sections revealed tightly interwoven mantles 15–50 $\mu$m thick with scattered erect surface hyphae. Hartig nets uniformly penetrated two to three cortical cell layers (Fig. 5).

*B. edulis* has long been recognized for its diverse host associations. Trappe (1962) lists species in 16 different genera of hosts including 6 members of the Pinaceae and 10 hardwood genera. Ectomycorrhizae have been synthesized in pure culture with *Pinus virginiana* Mill. (Vozzo and Hacskaylo 1961), *P. radiata* (Malajczuk and others 1982), *P. sylvestris* L. (Pachlewska 1968), and *Betula pendula* (Melin 1923); arbutoid mycorrhizae were synthesized on *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982). Thiers (1975) reports *Pinus* species to be the major associates of *B. edulis* in California but states that hardwoods should not be overlooked in this regard. We have collected *B. edulis* in equal abundance under species of *Tsuga, Pseudotsuga, Abies, Pinus,* and *Picea* in Oregon. Results from this study confirm the ability of *B. edulis* to form ectomycorrhizae with the diverse conifer flora of the Pacific Northwest.

*Cenococcum geophilum* Fr.

*C. geophilum* formed ectomycorrhizae with all hosts, usually abundantly. Ectomycorrhizae were characteristically jet black and anatomically as described by others (Melin 1927, Hatch 1934, Trappe 1971). Ectomycorrhizae were simple, 1–6 mm long, but became bifurcate on *Pinus* species and variably branched on Douglas-fir and up to 8 mm long. Cross sections revealed well-developed mantles 15–35 $\mu$m thick and Hartig nets on all hosts.

*C. geophilum* is the most ubiquitous and easily recognized ectomycorrhizal fungus. Trappe (1964) lists 122 associated host species including species in 6 genera of gymnosperms and 19 genera of angiosperms. More recent literature citations would bring the total associates to over 200 species including 12 additional genera (Trappe, unpublished compilation).

*Cortinarius pistorius* Schaeff. ex Fr.

*C. pistorius* only formed ectomycorrhizae with the three *Pinus* species and for each host the ectomycorrhizal structure was weakly developed. Diffuse white

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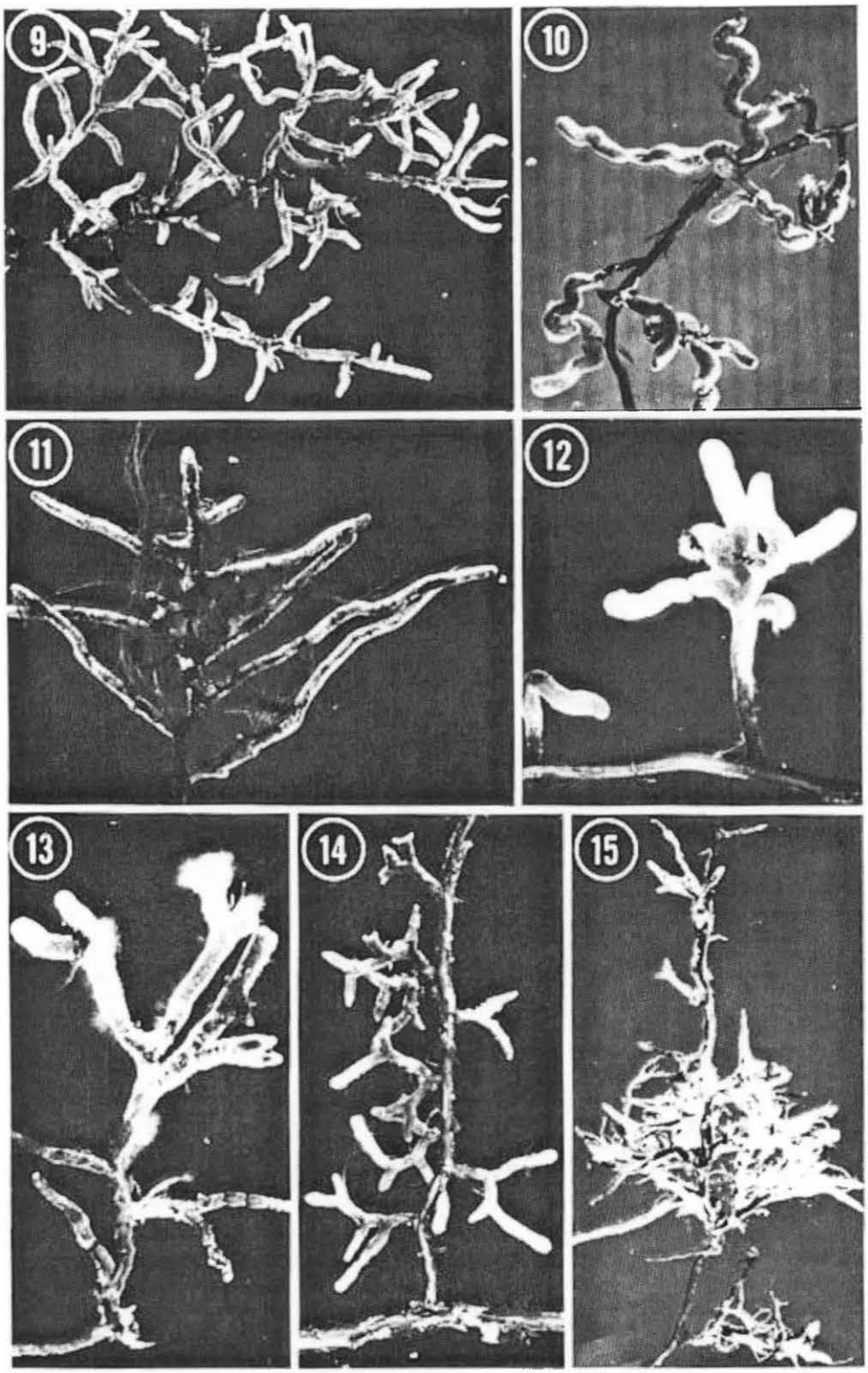
FIGURES 9–15. Synthesized ectomycorrhizae.

FIG. 9. *Amanita muscaria* + *Picea sitchensis*, ×2.6.
FIG. 10. *Amanita muscaria* + *Larix occidentalis*, ×3.9.
FIG. 11. *Astraeus pteridis* + *Pinus monticola*, ×3.2.
FIG. 12. *Boletus edulis* + *Larix occidentalis*, ×5.8.
FIG. 13. *Gastroboletus subalpinus* + *Pinus ponderosa*, ×5.8.
FIG. 14. *Gastroboletus subalpinus* + *Pinus monticola*, ×3.2.
FIG. 15. *Hysterangium separabile* + *Pinus contorta*, ×2.6.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mycelium sparsely sheathed the feeder roots. Ectomycorrhizae varied from cylindric to bifurcate, 2–8 mm long, but were not distinctly swollen. Cross sections showed loosely interwoven mantles 2–12 $\mu$m thick and weakly developed Hartig nets which irregularly penetrated only the outer cortical cell layer.

Although *Cortinarius* is believed to be the largest genus of ectomycorrhizal fungi (Smith 1971), little data is available on its mycorrhiza-forming abilities. Many species fail to grow or grow slowly in culture. *C. pistorius* profusely colonized the peat-vermiculite substrate, yet ectomycorrhizae remained weakly developed. It may well be that *C. pistorius* is only facultatively ectomycorrhizal, at least under these experimental conditions, and is specific to species of *Pinus* in this regard.

*Fuscoboletinus aeruginascens* (Secr.) Pom. & Smith

*F. aeruginascens* formed well-developed ectomycorrhizae with western larch, Douglas-fir, and Sitka spruce. Nearly all short roots of western larch and Douglas-fir were colonized. Ectomycorrhizae were white, occasionally with a pale pinkish-brown cast, and with a cottony to wooly tomentose surface; numerous thick, pinkish white translucent hyphal strands were noted. Western larch ectomycorrhizae branched prolifically forming large fanlike structures up to 20 mm across (Fig. 55). Douglas-fir and Sitka spruce ectomycorrhizae were mostly simple, 2–5 mm long for Sitka spruce and up to 9 mm long for Douglas-fir.

Cross sections revealed mantles to be tightly interwoven but with a loose outer surface. Douglas-fir mantles were 50–85 $\mu$m thick but other mantles were up to 125 $\mu$m on western larch (Fig. 6) and Sitka spruce (Fig. 7). Well-developed Hartig nets were prominent in each host, uniformly penetrating two to four cortical cell layers. Tannin layers developed in both western larch and Sitka spruce ectomycorrhizae although in Douglas-fir sections only a mild safranin staining of cortical cells was noted.

All *Pinus* spp. appeared superficially to bear abundant ectomycorrhizae. Short roots were well mantled and branched dichotomously, but the mantling mycelium was much looser than noted above and at times crustlike. Cross sections revealed primarily surface mycelium with only occasional isolated penetrations into cortex (Fig. 8). As in the *Alpova diplophloeus* syntheses, these roots had an overall unhealthy appearance with disruption of the tissue and often intense safranin staining of cortical cells. A few outer cells were also seen filled with hyphae.

Sporocarps of *F. aeruginascens* are always associated with *Larix* species (Smith and Thiers 1971), and numerous authors have viewed this as an ectomycorrhizal association (see Trappe 1962). This ectomycorrhizal relationship is now confirmed, but it was surprising to find it also able to form ectomycorrhizae with Douglas-fir and Sitka spruce; *F. aeruginascens* has also formed arbutoid mycor-

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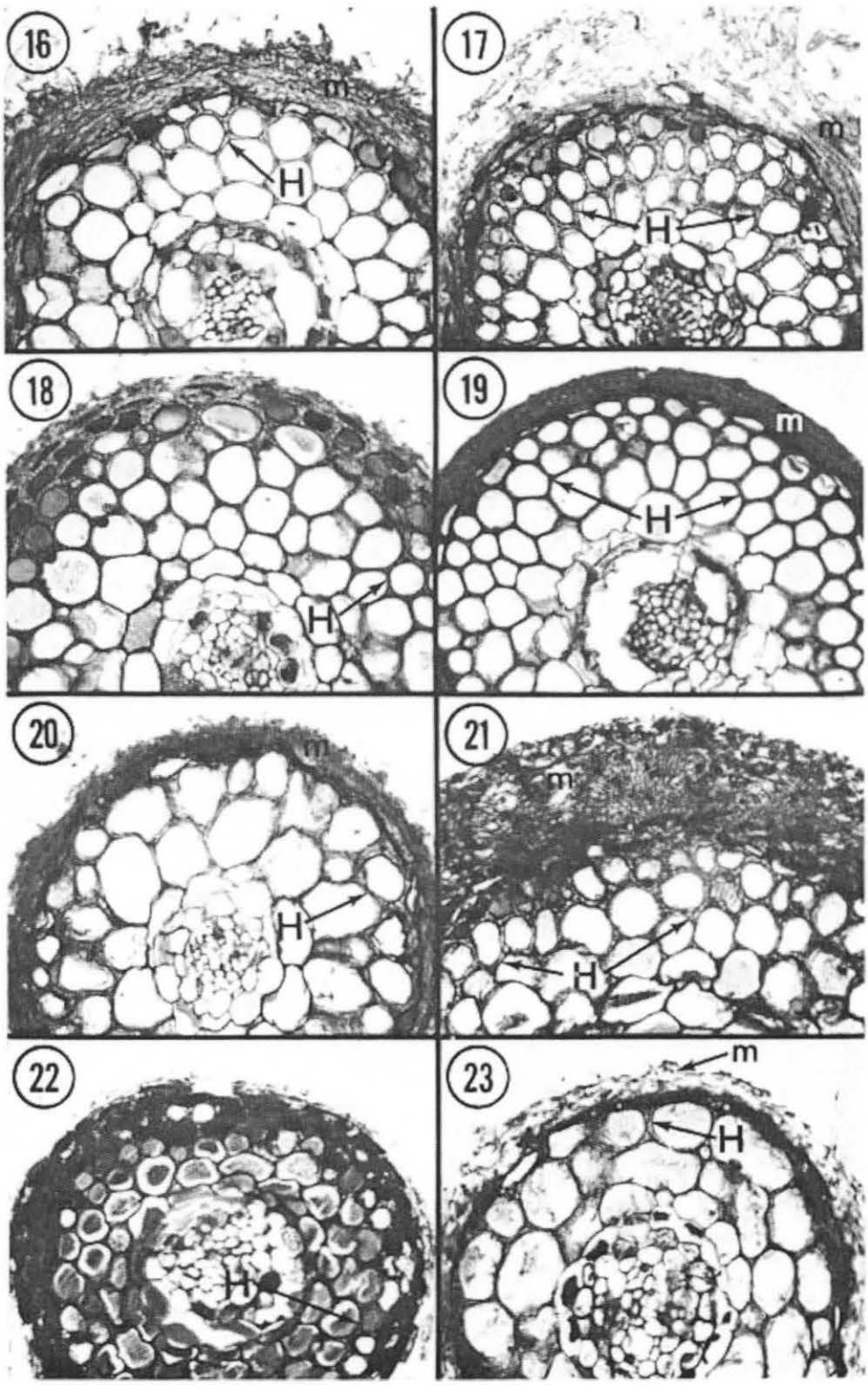
FIGURES 16–23. Cross sections of synthesized ectomycorrhizae. All figures ×170 (m, mantle; H, Hartig net).

FIG. 16. *Gastroboletus subalpinus* + *Pinus ponderosa*.
FIG. 17. *Hysterangium separabile* + *Tsuga heterophylla*.
FIG. 18. *Laccaria laccata* + *Pseudotsuga menziesii*.
FIG. 19. *Lactarius deliciosus* + *Tsuga heterophylla*.
FIG. 20. *Leccinum manzanitae* + *Pinus ponderosa*.
FIG. 21. *Melanogaster intermedius* + *Pseudotsuga menziesii*.
FIG. 22. *Paxillus involutus* + *Pseudotsuga menziesii*.
FIG. 23. *Paxillus involutus* + *Pinus monticola*.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rhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982). Although Trappe (1962) cites possible associations with *Pinus* species, *F. aeruginascens* appeared incompatible with the *Pinus* species used in this study.

*Gastroboletus subalpinus* Trappe & Thiers

*G. subalpinus* formed well-developed ectomycorrhizae on all *Pinus* spp.; only one Douglas-fir feeder root was colonized, but it too was well developed. Ectomycorrhizae were initially white to beige, becoming a pale grey-brown with age, with a felty appressed to smooth mantle surface. Individual ectomycorrhizae were noticeably swollen and succulent as described for *Boletus edulis* ectomycorrhizae. *Pinus* ectomycorrhizae were simple to mostly bifurcate and compoundly bifurcate, 2–10 mm long (Figs. 13 and 14). The one Douglas-fir ectomycorrhiza branched pinnately and was 11 mm long.

Cross sections of *Pinus* ectomycorrhizae showed variably thick mantles, 25–62 $\mu$m, composed of tightly interwoven hyphae with looser strands on the periphery. Hartig nets uniformly penetrated two to three cortical cell layers (Fig. 16). The mantle on Douglas-fir was similar in thickness but not as uniformly interwoven. The Hartig net was also less well developed, occasionally patchy, but easily discernable. A tannin layer also occurred on Douglas-fir.

*G. subalpinus* is an infrequently collected hypogeous secotoid bolete similar to species centered around *Boletus edulis* (Thiers and Trappe 1969). It fruits most commonly at high elevations in association with *Pinus albicaulis* Engelm. and *Abies* species with which Thiers and Trappe (1969) speculate it is ectomycorrhizal; they also add that other conifers of montane and subalpine forests may be involved. It has also formed arbutoid mycorrhizae in pure culture synthesis with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982).

*Hysterangium separabile* Zeller

*H. separabile* formed ectomycorrhizae with lodgepole pine, western white pine, western larch, and western hemlock. This very slow-growing fungus rarely colonized the entire peat-vermiculite substrate and was most often limited to dense mycelial patches or failed to grow entirely. This probably accounted for the low abundance of colonized short roots and uncompleted syntheses. Ectomycorrhizae were white and densely pubescent. On lodgepole pine, they were simple to bifurcate, 4–10 mm long, and often interconnected by large wefts of emergent mycelia and stout ropelike rhizomorphs (Fig. 15). Western white pine ectomycorrhizae remained simple, 1–2 mm long. The few colonized short roots of western larch and hemlock lacked the prolific external mycelial development and were mostly simple structures, 1–5 mm long.

Cross sections revealed mantles on all hosts to be loosely interwoven, 12–50 $\mu$m thick on western hemlock but a more uniform 50–60 $\mu$m on lodgepole pine (Fig. 17). Prominent tannin layers and considerable safranin staining of cortical cells were noted in lodgepole pine sections but did not impede uniform penetration of Hartig nets. Hartig nets were well developed in all other hosts.

*H. separabile*, a hypogeous basidiomycete in the Hysterangiaceae, commonly fruits in association with Douglas-fir in the Pacific Northwest (see photograph by Zak, 1973) but we have also collected it beneath *Pinus* spp. It has also been reported beneath *Carpinus* and *Fagus* spp. in Europe (Froidevaux and Schwarzel 1977) and formed arbutoid mycorrhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* in a previous study (Molina and Trappe 1982). Results from this study confirm its ability to form ectomycorrhizae with a wide range of hosts.

*Laccaria laccata* (Scop. ex Fr.) Bk. and Br.

*L. laccata* formed well-developed ectomycorrhizae with all hosts, often colonizing entire short root systems. Ectomycorrhizae were pale orange-brown with pale lavender tips when young and smooth. *Pinus* ectomycorrhizae developed typically into compound bifurcate structures, 1–7(–13) mm long (Fig. 25). Douglas-fir and western larch ectomycorrhizae were initially simple but became pinnate to variously branched, 1–10 mm long on Douglas-fir and up to 16 mm long on western larch. Western hemlock ectomycorrhizae remained cylindric but often elongate, up to 25 mm long (Fig. 24). Sitka spruce ectomycorrhizae were mostly simple club-shaped structures but occasionally became variably branched, 3–15 mm long.

Cross sections revealed well-developed, tightly interwoven mantles with appressed surfaces, averaging 25 $\mu$m thick. Hartig nets were prominent and uniformly developed on all hosts. Tannin layers occurred in Douglas-fir sections (Fig. 18).

Even though root systems bore abundant ectomycorrhizae, all hosts consistently showed stunted, nutrient deficient stems and needles for reasons unknown.

*L. laccata* is well known as a mycorrhizal fungus; Trappe (1962) lists it in association with seven genera of hosts. It has formed ectomycorrhizae in pure culture syntheses with several *Pinus* spp. (Trappe 1962, Marx and Davey 1969, Marx and Zak 1965, Pachlewski and Pachlewska 1974), Sitka spruce (Thomas and Jackson 1979), Douglas-fir (Stack and Sinclair 1975) and arbutoid mycorrhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Zak 1976, Molina and Trappe 1982). *L. laccata* is one of the more common agarics in conifer forest types in the Pacific Northwest. It has been effective in inoculation of several species of container-grown seedlings (Molina 1980a, Shaw and Molina 1980).

*Lactarius deliciosus* (L. ex Fr.) S. F. Gray

*L. deliciosus* formed numerous, conspicuously smooth, swollen, and often elongate ectomycorrhizae with all hosts (Fig. 28). Ectomycorrhizae were pale orange-brown with occasional green stains as also seen on sporocarps. *Pinus* ectomycorrhizae developed into coralloid structures, 2–10(–20) mm across. Remaining hosts showed primarily elongate cylindric ectomycorrhizae that on Douglas-fir became variably branched and up to 25 mm long.

Cross sections revealed classical ectomycorrhizal anatomy with tightly interwoven mantles with fine hyphae appressed on the surface. Mantles ranged in thickness from 10–25 $\mu$m on *Pinus* spp. to 25–50 $\mu$m on Douglas-fir. Hartig nets uniformly penetrated 2–4 cortical cell layers (Fig. 19).

*L. deliciosus* fruits throughout the Pacific Northwest, especially in association with Sitka spruce along the coast and with *Abies, Pseudotsuga,* and *Pinus* spp. in interior forest communities. In pure culture syntheses, it has formed ectomycorrhizae with several *Pinus* species (Trappe 1962, Riffle 1973) and *Picea abies* (Melin 1925a), and arbutoid mycorrhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982).

*Leccinum manzanitae* Thiers

*L. manzanitae* formed ectomycorrhizae with lodgepole and ponderosa pine as well as Douglas-fir, western hemlock, and Sitka spruce. It grew slowly through the substrate and thus colonized relatively few feeder roots. Like *B. edulis* ectomycorrhizae, these were swollen, and white with a finely pubescent to occasionally smooth-appressed surface. Pine ectomycorrhizae were simple to compact-bifurcate, 3–6 mm long (Fig. 27). Ectomycorrhizae of all other hosts were

simple and 1–5 mm long. Sitka spruce mycorrhizae were more swollen at the base, narrowing to an uncolonized tip.

Cross sections revealed tightly interwoven mantles with occasional loose patches of mycelium on the surface; mantles varied in thickness from 5–50 $\mu$m on *Pinus* spp. and Douglas-fir to a more uniform ca. 50 $\mu$m on Sitka spruce and western hemlock. Hartig nets developed well on all hosts, especially on *Pinus* spp. where they widely separated adjacent cortical cells (Fig. 20). Tannin layers developed in *Pinus* ectomycorrhizae.

*L. manzanitae* has been reported fruiting only in association with the ericaceous hosts *Arbutus menziesii* or *Arctostaphylos* spp. along the coast of California and Oregon (Thiers 1975) and has been shown to form arbutoid mycorrhizae with these hosts (Molina and Trappe 1982). It was thus surprising to find it able to form ectomycorrhizae with such a wide range of other hosts. The ericaceous hosts, however, are often understory or neighboring components of coastal forests. The isolate of *L. manzanitae* used in this study was from a sporocarp found directly beneath lodgepole pine in a prostrate mat of *Arctostaphylos uva-ursi*.

*Melanogaster intermedius* Zeller & Dodge

*M. intermedius* formed prolific ectomycorrhizae with all hosts. Ectomycorrhizae were golden brown, becoming dark brown with age and with an appressed surface and abundant dark brown, thickened hyphal strands (Fig. 30). *Pinus* ectomycorrhizae developed into compact coralloid structures 1–5 mm across. Douglas-fir, western larch, and Sitka spruce ectomycorrhizae were cylindric to more commonly pinnate and compoundly pinnate or occasionally variably branched (on western larch) and up to 15 mm long. Western hemlock ectomycorrhizae remained simple, 2–5 mm long.

Cross sections revealed well-developed mantles and Hartig nets for all hosts. Mantles were mostly tightly interwoven with occasional loose surface patches, and averaged ca. 35 $\mu$m thickness on most hosts but up to 100 $\mu$m thick on Douglas-fir (Fig. 21). Hartig nets uniformly penetrated 2–4 cortical cell layers. Tannin layers were present on most hosts; and on Douglas-fir, a deeply safranin-stained ring of collapsed outer cells circumscribed the ectomycorrhizae.

*Melanogaster* is a genus of hypogeous Basidiomycetes phylogenetically related to *Rhizopogon*, but its mycorrhizal status was heretofore unconfirmed by pure culture synthesis. Although *Melanogaster* spp. are regularly collected in the Pacific Northwest, the taxonomy of the genus is too poorly known to permit dependable identifications of most taxa. Reliable host range data are thus not yet available. This particular isolate was collected beneath Douglas-fir and *Quercus garryana* Dougl. Probable ectomycorrhizal associations with *Fagus sylvatica* L. and *Quercus* spp. in France have been reported by Froidevaux and Schwarzel (1977). This is the first report of a hypogeous Basidiomycete able to form such

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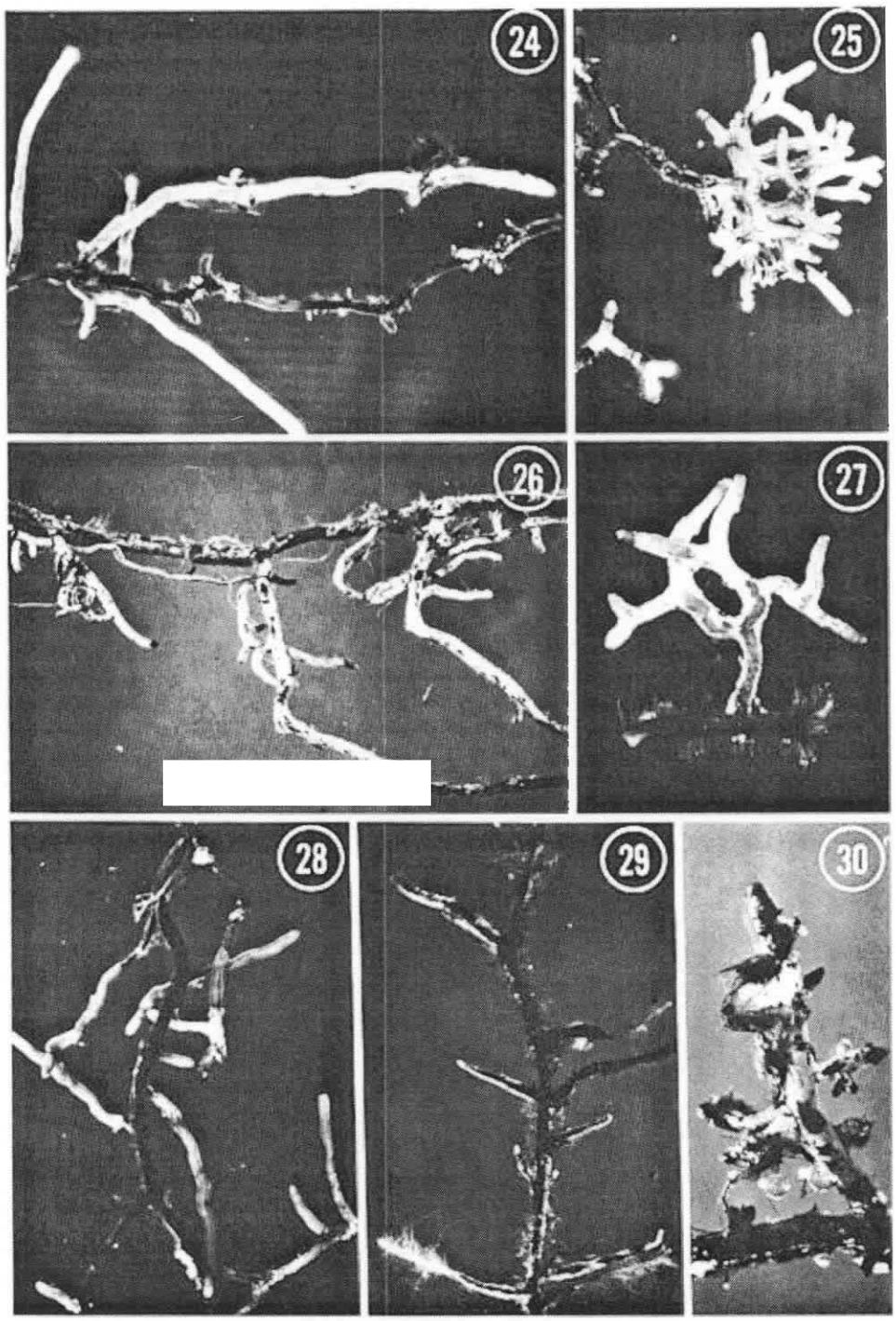
FIGURES 24–30. Synthesized ectomycorrhizae.

FIG. 24. *Laccaria laccata* + *Tsuga heterophylla*, ×2.6.
FIG. 25. *Laccaria laccata* + *Pinus monticola*, ×3.9.
FIG. 26. *Paxillus involutus* + *Larix occidentalis*, ×3.2.
FIG. 27. *Leccinum manzanitae* + *Pinus contorta*, ×6.8.
FIG. 28. *Lactarius deliciosus* + *Tsuga heterophylla*, ×6.8.
FIG. 29. *Rhizopogon fuscorubens* + *Picea sitchensis*, ×2.6.
FIG. 30. *Melanogaster intermedius* + *Pseudotsuga menziesii*, ×4.8.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prolific ectomycorrhizae with a wide range of diverse hosts. It merits testing for inoculation of forest seedling nurseries.

*Paxillus involutus* (Batsch ex Fr.) Fr.

*P. involutus* formed abundant ectomycorrhizae with all hosts. Ectomycorrhizae were initially white but soon became pale brown to golden brown with occasional thin wefts of emergent hyphae and hyphal strands. Individual ectomycorrhizae were strikingly narrow-cylindric. *Pinus* ectomycorrhizae were mostly simple to bifurcate but occasionally developed into complex coralloid structures up to 9 mm across. Douglas-fir and western larch ectomycorrhizae became variably branched and up to 20 mm long on western larch (Fig. 26). Western hemlock and Sitka spruce ectomycorrhizae were primarily simple, occasionally branching, 1–7 mm long for Sitka spruce and up to 12 mm long for western hemlock. Sclerotia as described by Laiho (1970) were noted adhering to ectomycorrhizae and throughout the peat-vermiculite substrate.

Although the short roots appeared well mantled, cross sections revealed fragmentary and loosely interwoven mantles ranging from absent in places to 25 $\mu$m thick. Uniform Hartig net penetrations were seen in Douglas-fir, western larch, and western white pine cross sections (Figs. 22 and 23). Remaining hosts had more scattered or localized Hartig nets which were well developed but not uniform in penetration. On most hosts, outer cortical cells stained strongly with safranin; in Douglas-fir this appeared as two deeply stained outer cortical cell layers circumscribing the ectomycorrhiza, although Hartig net penetration was not restricted.

*P. involutus* received intensive study by Laiho (1970) in Europe. He found it to form ectomycorrhizae under aseptic and semi-aseptic conditions with species of *Picea, Pseudotsuga, Alnus, Betula, Tsuga,* and also listed probable associations with *Fagus, Pyrus, Quercus, Rhamnus, Salix,* and *Populus*. It is also capable of forming ectomycorrhizae with several species of *Eucalyptus* (Malajczuk and others 1982), as well as arbutoid mycorrhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982). We rarely observe *P. involutus* sporocarps in forest habitats, but it is ubiquitous in filbert (*Corylus avellana* L.) orchards and beneath ornamental *Betula* spp. in the Willamette Valley of Oregon.

The ectomycorrhizal anatomy described above is apparently typical of *P. involutus* colonizations. Laiho (1970) and Pachlewski and Pachlewska (1974) both report fragmentary mantles and incomplete Hartig net development. Molina (1979, 1981) found it to only rarely penetrate intercellularly in *Alnus* ectomycorrhizae and noted intense safranin staining in outer cortical cells. Pachlewski and Pachlewska (1974) noted a brown discoloration in cortical cells of *Pinus sylvestris* + *P. involutus* ectomycorrhizae. *P. involutus* is a ectomycorrhizal fungus worthy of study regarding the phenolic responses it appears to elicit from numerous hosts.

*Pisolithus tinctorius* (Pers.) Coker & Couch

*P. tinctorius* formed prolific ectomycorrhizae with all hosts. Ectomycorrhizae were bright yellow, becoming golden brown with age. Mantle surfaces were loosely tomentose with abundant emerging hyphal wefts and strands. *Pinus* mycorrhizae branched dichotomously and were 2–10 mm long. Douglas-fir, western larch, and Sitka spruce formed simple to pinnate and variously branched structures up to 20 mm long. Sitka spruce ectomycorrhizae were noticeably less swollen than those of other hosts and produced few hyphal strands. Western hemlock ectomycorrhizae remained simple, 2–10 mm long.

Cross sections of all ectomycorrhizae showed classical ectomycorrhizal anatomy. Mantles were 25–27 μm thick, tightly interwoven at the root surface, and loosely intertwined at the mantle surface. Hartig nets developed uniformly and penetrated 2–3 cortical cell layers.

*P. tinctorius* is cosmopolitan, has been experimentally proven to form ectomycorrhizae with 48 tree species, and observed in association with 23 additional host species (Marx 1977), including species of *Abies, Betula, Carya, Eucalyptus, Pinus, Populus, Pseudotsuga, Quercus, Salix,* and *Tsuga. Larix* can now also be added to this list of host genera, as can *Arbutus* and *Arctostaphylos* from a previous study (Molina and Trappe 1982). Its diverse host range and ability to function as viable and effective inoculum has made *Pisolithus tinctorius* a prime candidate for use in nursery operations (Marx 1977).

*Rhizopogon cokeri* A. H. Smith

*R. cokeri* formed ectomycorrhizae only with the three *Pinus* spp. These ectomycorrhizae were initially white but soon turned to bright lemon yellow, eventually dulling to a yellowish brown. Mantles were loosely tomentose with emerging wefts of yellow mycelium. The ectomycorrhizae varied from occasionally simple to mostly tightly compact bifurcate and coralloid, 0.5–5 mm long.

Cross sections showed mantles 13–100 μm thick and more or less tightly interwoven with loose surface mycelium. Hartig nets uniformly penetrated two cortical cell layers (Fig. 31). A few western hemlock short roots were mantled, but cross sections showed this mycelium to be only surface growth. Underlying cortical cells were noticeably disrupted and deeply safranin stained.

*R. cokeri* showed strong preference for *Pinus* in this study. The isolate specimen was collected in a mixed *Pinus-Pseudotsuga* forest, and it has also been reported from beneath pine in Idaho by Smith and Zeller (1966). In Oregon, however, we have most commonly found *R. cokeri* in mycorrhizal association with mountain hemlock (*Tsuga mertensiana*); the sporocarp peridium, tuberculate ectomycorrhizae, and rhizomorphs of *R. cokeri* are uniquely bright yellow and have been easily traced to mountain hemlock roots (Zak 1973).

*Rhizopogon fuscorubens* A. H. Smith

*R. fuscorubens* ectomycorrhizae were best developed on lodgepole and ponderosa pines. These ectomycorrhizae were white to tan with tomentose surface; emergent mycelium and coarse pale brown hyphal strands were abundant. Ectomycorrhizae developed were mostly compact bifurcate to coralloid, 0.5–3 mm long. Cross sections showed mantles 12–30 μm thick and tightly interwoven at the root surface but more loosely organized at the periphery. Hartig nets were prominent, widely separating cortical cells, and uniformly penetrating two to three cell layers (Fig. 32).

Ectomycorrhizae were also noted in western larch and Sitka spruce syntheses but these appeared more superficial. Mantles were noticeably more sparse and loose in structure than on *Pinus* spp., especially on western larch where colonized short roots were least numerous. Sitka spruce ectomycorrhizae remained simple (Fig. 29), 2–7 mm long; whereas western larch elements became variously branched and up to 10 mm long. Cross sections of Sitka spruce ectomycorrhizae showed mantles well developed as on *Pinus*, 12–50 μm thick. The Hartig net was uneven in penetration, occasionally occurring in isolated patches; but cortical cells were not disrupted (Fig. 33). Western larch ectomycorrhizae, in contrast, had irregular mantles 15–42 μm thick and varying from tightly interwoven mycelial patches to a much looser structure. Hartig nets were prominent in some

serial sections, but in others irregularly penetrating and disrupting the cortex (Fig. 34).

*R. fuscorubens* has been infrequently collected, always beneath coastal lodgepole pine. Sitka spruce, however, is commonly mixed with lodgepole pine in these coastal habitats. *R. fuscorubens* is closely related to *R. ochraceorubens* (Smith and Zeller 1966), which also shows strong ectomycorrhizal associations with *Pinus* (Molina 1980b).

*Rhizopogon occidentalis* Zeller & Dodge

*R. occidentalis* formed classical ectomycorrhizae with lodgepole and western white pines. These were white to creamy white, finely tomentose with occasional smooth patches and stout hyphal strands. Most ectomycorrhizae developed into compact bifurcate and coralloid forms, 1–3 mm across. Cross sections showed mantles 25–70 $\mu$m thick and tightly interwoven with a few loose outer hyphae. Hartig nets were prominent, widely separating and uniformly penetrating three cortical cell layers (Fig. 35).

Ectomycorrhizal status of the few Sitka spruce short roots colonized appeared questionable. They remained simple and 2–4 mm long. Mantles were thin with occasional protruding root hairs, in cross section sparse and loosely appressed. Prominent Hartig nets penetrated to the endodermis, however, widely separating and slightly disrupting the cortical cells (Fig. 36).

*R. occidentalis* is among the more common *Rhizopogon* spp. associated with pines throughout the Pacific Northwest. This study confirms its preference for pine hosts. It is especially prevalent beneath coastal lodgepole pine, where it can often contact roots of intermixed Sitka spruce.

*Rhizopogon vinicolor* A. H. Smith

*R. vinicolor* ectomycorrhizae developed best on Douglas-fir as described by Zak (1971): initially white, becoming pale brown to dark brown with age, simple to pinnate, 2–15 mm long (Fig. 39). Cross sections showed well-developed mantles 20–45 $\mu$m thick, uniformly penetrating Hartig nets and prominent tannin layers.

For all other hosts, colonizations were either unusual in one or more characters or were totally superficial. Only a few western hemlock short roots were colonized. These appeared mostly as simple swellings 2–4 mm long, with a thin, loosely appressed mantle. Although cross sections showed the thin mantle to range from absent to 12 $\mu$m thick, Hartig nets developed well and uniformly penetrated 2–3 cortical cell layers deep (Fig. 37). Colonized roots of western larch were white with darker underlying grey mycelium and elongated into variably branched structures 2–10 mm long. Cross sections revealed a fairly well-developed Hartig net 2–3 cortical cell layers deep (Fig. 38). Cortical cells adjacent to

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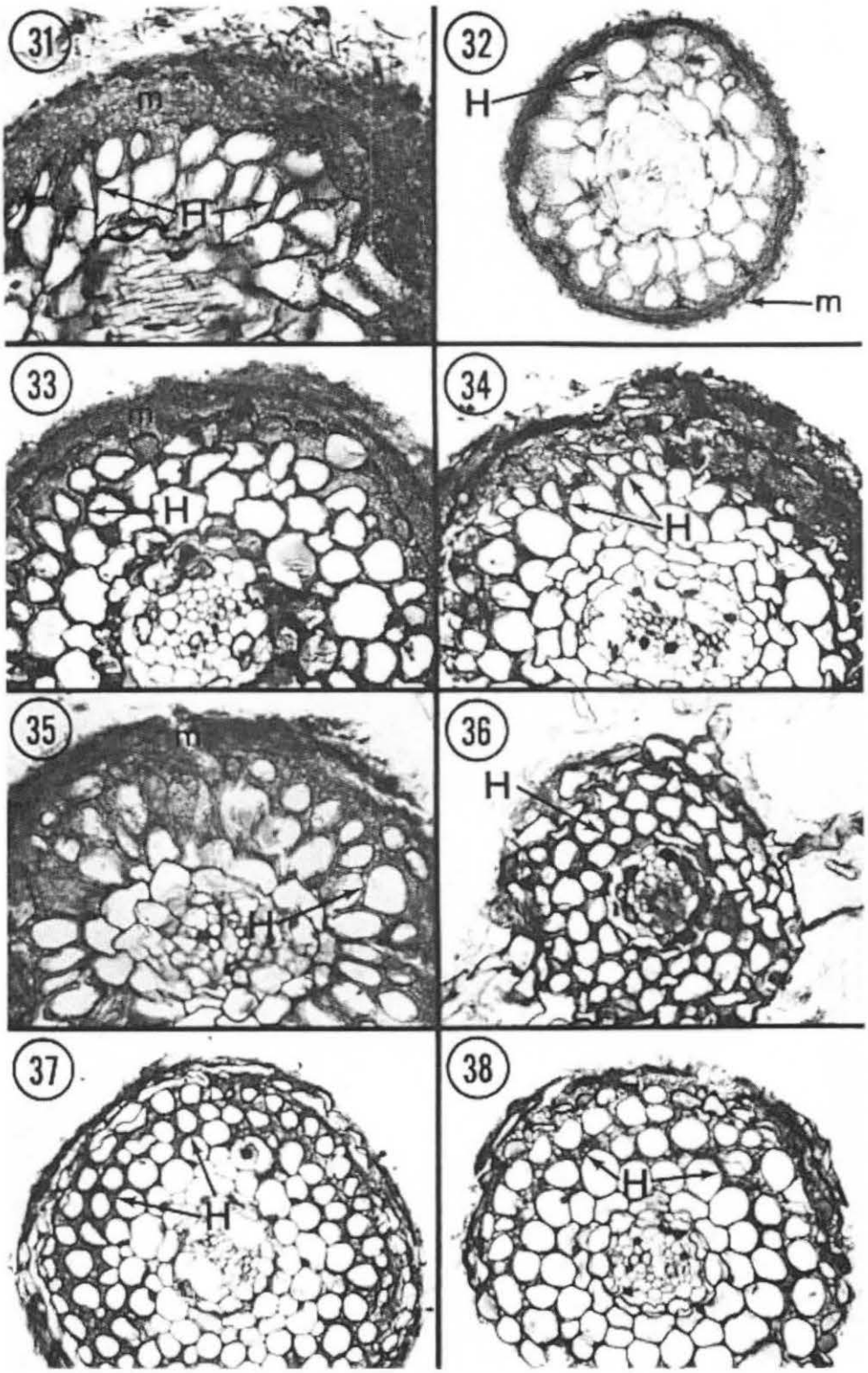
FIGURES 31–38. Cross sections of synthesized ectomycorrhizae. All figures ×170 (m, mantle; H, Hartig net).

FIG. 31. *Rhizopogon cokeri* + *Pinus ponderosa*.
FIG. 32. *Rhizopogon fuscorubens* + *Pinus contorta*.
FIG. 33. *Rhizopogon fuscorubens* + *Picea sitchensis*.
FIG. 34. *Rhizopogon fuscorubens* + *Larix occidentalis*.
FIG. 35. *Rhizopogon occidentalis* + *Pinus contorta*.
FIG. 36. *Rhizopogon occidentalis* + *Picea sitchensis*.
FIG. 37. *Rhizopogon vinicolor* + *Tsuga heterophylla*.
FIG. 38. *Rhizopogon vinicolor* + *Larix occidentalis*.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the sparse mantling hyphae, however, were deeply safranin stained. Colonized Sitka spruce roots were elongate cylindric, 3–7 mm long. Cross sections revealed a thick, loosely interwoven mantle, but the intercellular hyphae, although easily discernible, did not penetrate uniformly and completely disrupted the cortex (Fig. 44).

Short roots of lodgepole pine and, to a lesser extent, ponderosa pine were abundantly mantled and dichotomously branched, often compoundly so. Cross sections revealed only surface mantling (Fig. 45). Numerous outer cortical cells had collapsed and stained deeply with safranin, at times forming a ring through which the fungus rarely penetrated. On western white pine, however, a few short roots became noticeably swollen, although the mantling mycelium remained sparse to absent. Definite Hartig nets developed in these roots and penetrated fairly uniformly.

Sporocarps and tuberculate ectomycorrhizae of *Rhizopogon vinicolor* have been always associated with Douglas-fir in the field (Smith and Zeller 1966, Trappe 1965). Thus, it's not surprising that the most well-formed ectomycorrhizae developed with Douglas-fir. Western hemlock commonly accompanies Douglas-fir in temperate rain forests, so it would seem likely these hosts might share mutual symbionts. Yet, under these experimental conditions, minimal ectomycorrhiza development occurred on the hemlock. Molina (1980b) found numerous other Douglas-fir-specific *Rhizopogon* spp. to express similar minimal ectomycorrhizal colonization of western hemlock and to cause similar host reaction on lodgepole pine roots in pure culture syntheses. Using scanning electron microscopy, Mexal and others (1979) also found *R. vinicolor* to be an unsuitable symbiont for lodgepole pine; they report considerable forking of feeder roots, but no true mantle or Hartig net, and abnormal cortical cell development at the short root base.

*Scleroderma hypogaeum* Zeller

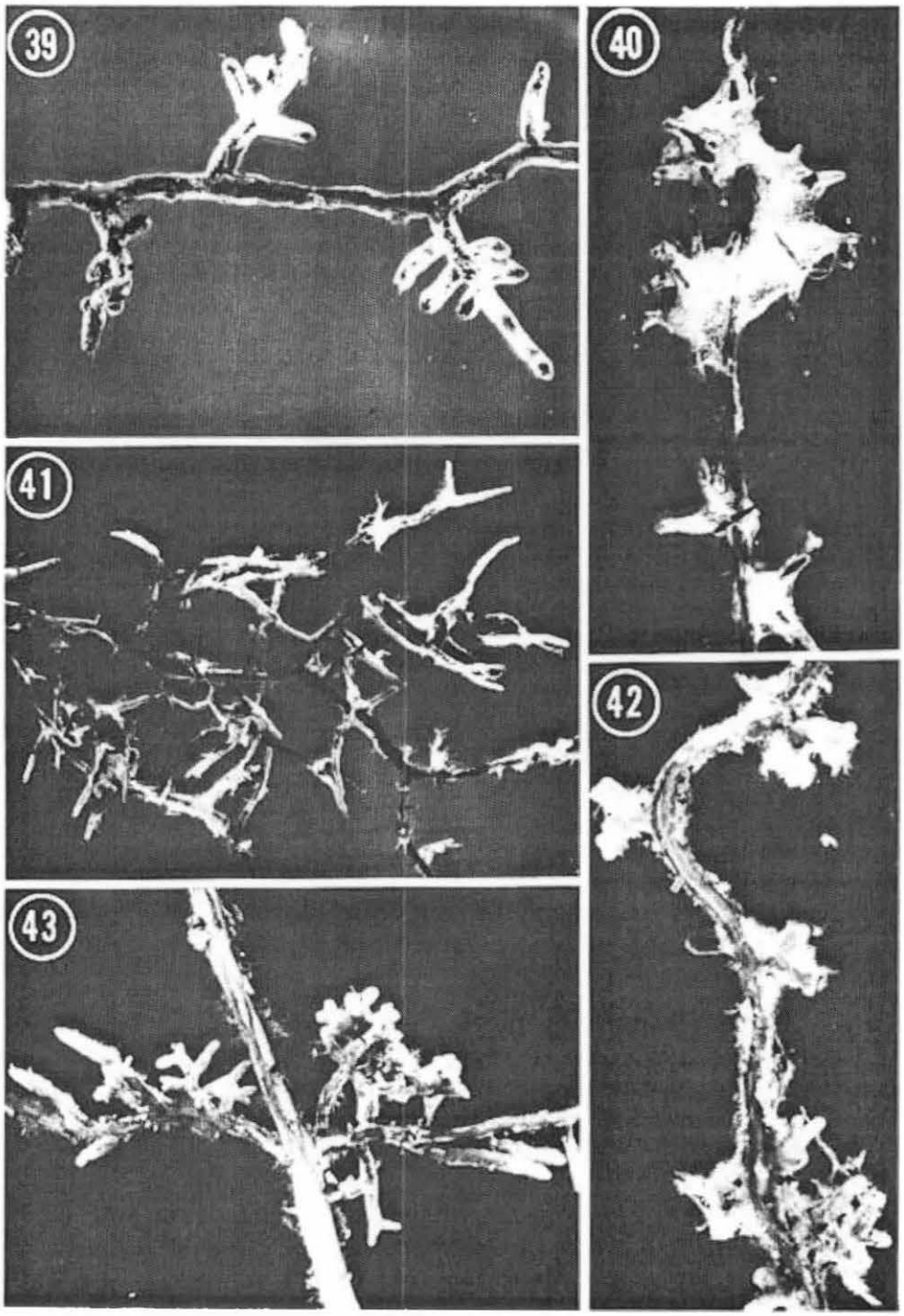
*S. hypogaeum* formed numerous well-developed ectomycorrhizae with all hosts. Ectomycorrhizae were bright white with mostly tomentose but occasional appressed mantle surfaces. Prolific surface mycelial wefts and occasional thick white hyphal strands often dominated their overall appearance (Figs. 40 and 41). *Pinus* ectomycorrhizae became bifurcate, 1–8 mm long (Fig. 40). Douglas-fir and western larch mycorrhizae varied from simple to variously branched, 2–10 mm long. Western hemlock and Sitka spruce ectomycorrhizae remained mostly simple, 2–7 mm long (Fig. 41).

Cross sections of all colonized roots showed classical ectomycorrhiza anatomy. Mantles were 12–60 $\mu$m thick and loosely interwoven, and Hartig nets uniformly penetrated 2–3 cortical cell layers. In western larch ectomycorrhizae, Hartig nets penetrated entirely to the endodermis and widely separated adjacent cortical cells.

Little data are available on the ectomycorrhizal host potential of *Scleroderma* spp. Our collection of *S. hypogaeum* was from beneath coastal lodgepole pine. Guzmán (1970) also reports its association with species of *Tsuga, Picea,* and *Pseudotsuga* in Oregon and with *Abies* spp. at high elevations in Central Mexico. This study confirms its ability to form ectomycorrhizae with diverse conifers. *S. hypogaeum* has also formed arbutoid mycorrhizae with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Molina and Trappe 1982) and limited ectomycorrhizae with *Alnus rubra* (Molina 1979).

*Suillus brevipes* (Peck) O. Kuntze

*S. brevipes* formed ectomycorrhizae with all hosts except western hemlock, with best development on *Pinus* spp. (Fig. 42). Ectomycorrhizae were initially white,




FIGURES 39–43. Synthesized ectomycorrhizae.

FIG. 39. *Rhizopogon vinicolor* + *Pseudotsuga menziesii*, ×4.3.
FIG. 40. *Scleroderma hypogaeum* + *Pinus contorta*, ×5.
FIG. 41. *Scleroderma hypogaeum* + *Picea sitchensis*, ×2.6.
FIG. 42. *Suillus brevipes* + *Pinus monticola*, ×5.3.
FIG. 43. *Suillus cavipes* + *Pinus monticola*, ×3.7.

becoming pale brown with age with a slight pinkish tinge; surfaces were usually densely tomentose with emergent thickened hyphal strands. Nearly all *Pinus* short roots were colonized and developed into large coralloid clusters up to 10 mm across. Douglas-fir short roots were also abundantly colonized, but individual ectomycorrhizae remained as stout cylinders 2–7 mm long (Fig. 53). Western larch short roots were colonized sparsely and often appeared only as basal swellings 1–5 mm long, with a protruding, uncolonized tip bearing root hairs. Even fewer Sitka spruce roots were colonized and these also appeared as basal swellings.

Cross sections of both *Pinus* and Douglas-fir roots showed classical ectomycorrhiza anatomy (Figs. 46 and 47). Mantles were 15–50 $\mu$m thick, mostly tightly interwoven with loose peripheral hyphae. Hartig nets uniformly penetrated 2–3(–4) cortical cell layers. Western larch mantles were fragmentary and thinner, from absent to 30 $\mu$m thick, but Hartig nets regularly penetrated 1–2 cortical cell layers. Sitka spruce differed in having thick mantles (up to 75 $\mu$m), which totally engulfed outer cortical cells and merged into a Hartig net which widely separated adjacent cells (Fig. 48). Western hemlock was sparsely colonized with only infrequent and scattered intercellular penetrations.

*S. brevipes* has been reported fruiting only under pines in California (Thiers 1975), an association previously confirmed by pure culture syntheses (Doak 1934, Grand 1968, Suggs and Grand 1972). We also find it common in pure stands of *Tsuga mertensiana* in Oregon. We did not expect it to form ectomycorrhizae with Douglas-fir, especially since they did not form in Grand's (1968) pure culture syntheses of that host-fungus combination. The weaker ectomycorrhizal development on western larch and Sitka spruce leaves their natural association open to question.

*Suillus cavipes* (Opat.) Smith & Thiers

*S. cavipes* formed well-developed ectomycorrhizae with western larch, Douglas-fir, western white pine, and to a lesser extent with lodgepole pine and western hemlock. Ectomycorrhizae were initially white to tan, becoming grey brown with age. Mantle surfaces were mostly loosely tomentose but occasionally appressed or crustlike. Western larch ectomycorrhizae showed greatest elongation varying from simple to irregularly pinnate structures 4–12 mm long. Douglas-fir remained simple, 1–9 mm long, but were noticeably swollen and often stubby. Western white pine developed typical pine ectomycorrhizae, well swollen and compound-bifurcate, 2–8 mm long (Fig. 43); fewer lodgepole pine roots became ectomycorrhizal and were less swollen and shorter, 2–4 mm long. The few western hemlock

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FIGURES 44–51. Cross sections of synthesized ectomycorrhizae and colonized feeder roots. All figures ×170 (m, mantle; H, Hartig net).

FIG. 44. *Picea sitchensis* colonized by *Rhizopogon vinicolor*. Note the heavy mantle colonization, disruption of the cortex and lack of true Hartig net.

FIG. 45. *Pinus contorta* colonized by *Rhizopogon vinicolor*. Note primarily only surface mycelium, no Hartig net, and disrupted outer cortex.

FIG. 46. *Suillus brevipes* + *Pinus ponderosa* ectomycorrhiza.

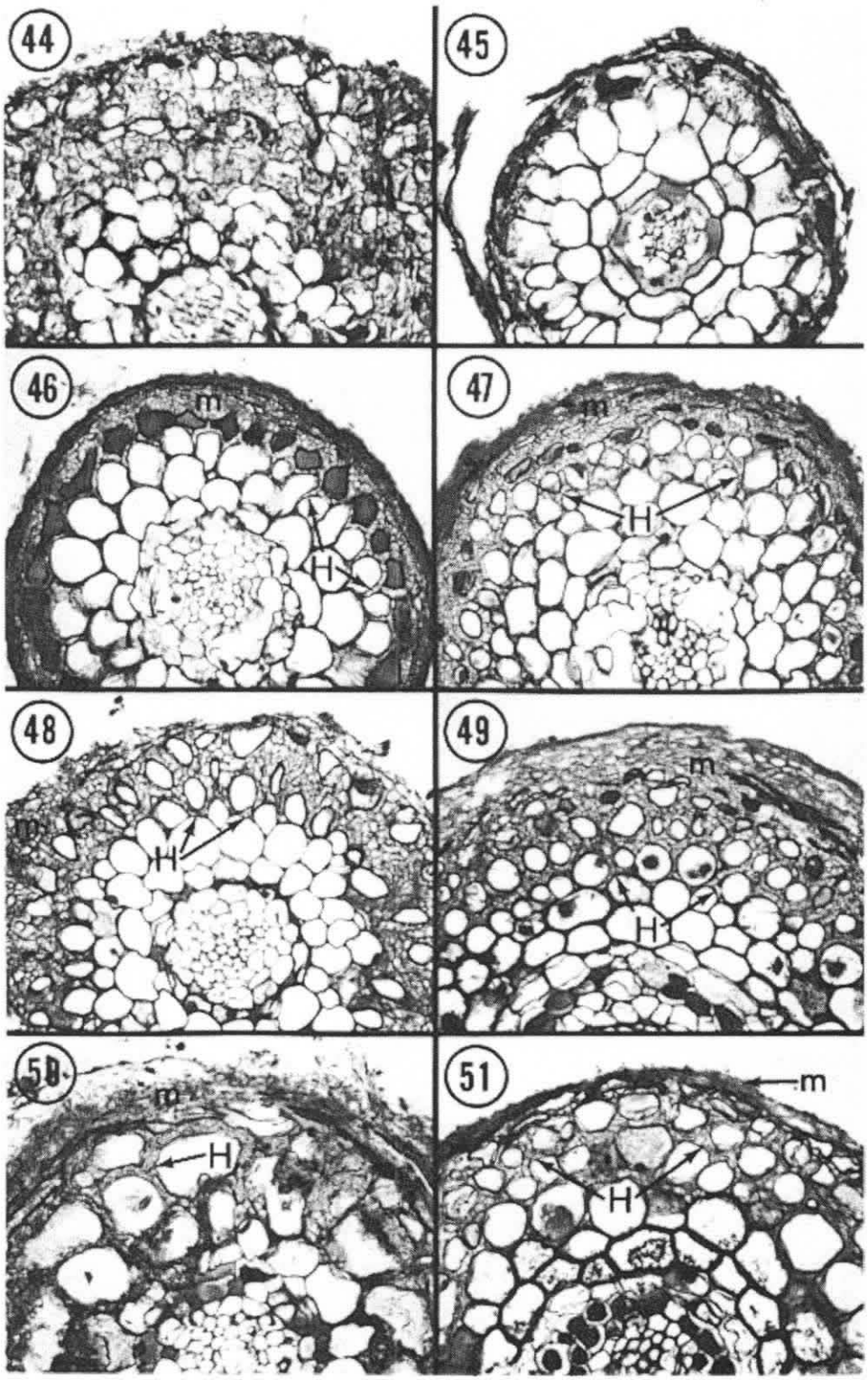
FIG. 47. *Suillus brevipes* + *Pseudotsuga menziesii* ectomycorrhiza.

FIG. 48. *Suillus brevipes* + *Picea sitchensis* ectomycorrhiza.

FIG. 49. *Suillus cavipes* + *Pseudotsuga menziesii* ectomycorrhiza.

FIG. 50. *Suillus cavipes* + *Pinus monticola* ectomycorrhiza.

FIG. 51. *Suillus grevillei* + *Pseudotsuga menziesii* ectomycorrhiza.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mycorrhizae remained simple, 1–3 mm long and appeared mostly swollen at the base.

Western larch, Douglas-fir, and western hemlock ectomycorrhizae were similar in cross sections; the mantles were 50–100 $\mu$m thick and mostly tightly interwoven with looser outer hyphae. Hartig nets uniformly penetrated three to four cortical cell layers, often widely separating adjacent cells (Fig. 49). Well-developed Hartig nets also formed on both lodgepole and western white pines (Fig. 50), but on lodgepole pine mantles varied from absent to 10 $\mu$m in thickness and a layer of outer cortical cells stained prominently with safranin.

*S. cavipes* constantly fruits under *Larix* spp. in the field (Smith and Thiers 1971) and presumed by many to be a host-specific ectomycorrhizal symbiont (see Trappe 1962). This association is confirmed but well-developed ectomycorrhizae also formed on other hosts, especially Douglas-fir and western white pine. We find *S. cavipes* associated with *Larix occidentalis* in stands typically mixed with Douglas-fir and various pine species. Natural ectomycorrhizal formation with these other hosts is thus quite possible.

*Suillus grevillei* (Klotzsch) Sing.

*S. grevillei* formed well-developed and abundant ectomycorrhizae with western larch and Douglas-fir. Ectomycorrhizae were white, densely tomentose, remaining cylindric on Douglas-fir, and 2–9 mm long (Fig. 54) but becoming variously branched on western larch and up to 10 mm long. Cross sections of ectomycorrhizae of both hosts showed mostly tightly interwoven mantles with loose mycelium on the surface, 15–30 $\mu$m thick on Douglas-fir, and 25–40 $\mu$m thick on western larch. Hartig nets regularly penetrated 2 to 4 cortical cell layers (Fig. 51).

Ponderosa and lodgepole pines were also colonized, the ponderosa more than the lodgepole (Figs. 56 and 57). These branched dichotomously and were slightly swollen. Surface mycelium appeared more appressed to crustose and sparse rather than densely tomentose. Cross sections confirmed thin appressed mantles and undeniable intercellular penetrations. Regular, uniform Hartig nets, however, were not evident; and some cortical cells stained with safranin (Fig. 60).

*S. grevillei* is generally reported to fruit only under *Larix* spp. Melin (1922) early demonstrated its ectomycorrhizal relationship with *Larix* and repeatedly emphasized the specific nature of the association. This study confirms for the third time, however, that *S. grevillei* can form ectomycorrhizae with Douglas-fir in pure culture (Grand 1968, Linnemann 1971). The other "larch specific" symbionts, *Fuscoboletinus aeruginascens* and *Suillus cavipes*, also formed abundant ectomycorrhizae with Douglas-fir. Douglas-fir and western larch may share some common compatibility towards such specialized ectomycorrhizal fungi.

Natural ectomycorrhizal associations with *Pinus* spp. remain doubtful, although it has been reported to fruit in association with *Pinus sylvestris* (Schwitzer

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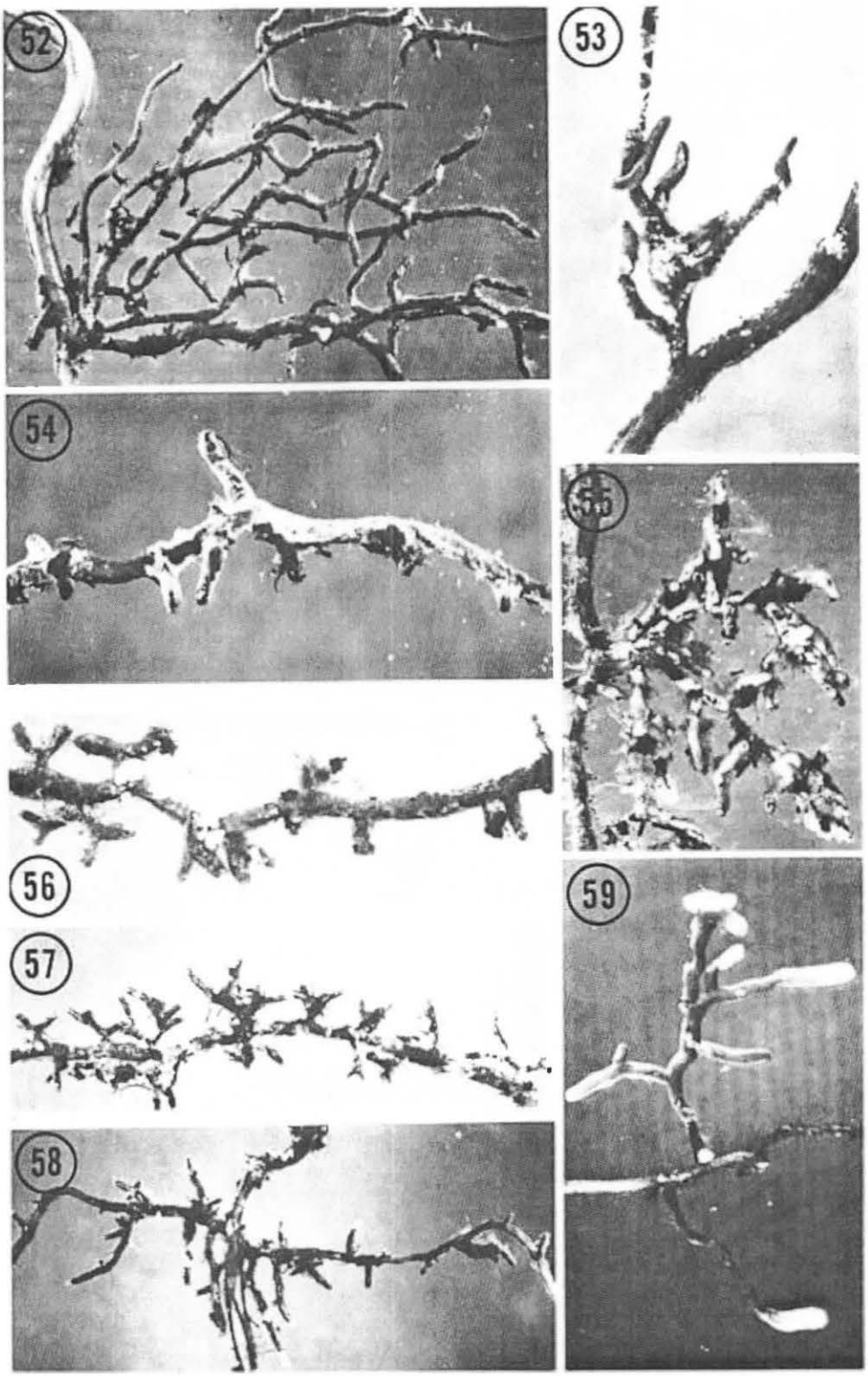
FIGURES 52–59. Synthesized ectomycorrhizae and questionable root colonizations.

FIG. 52. *Suillus lakei* + *Larix occidentalis* ectomycorrhizae, ×3.2.
FIG. 53. *Suillus brevipes* + *Pseudotsuga menziesii* ectomycorrhizae, ×4.3.
FIG. 54. *Suillus grevillei* + *Pseudotsuga menziesii* ectomycorrhizae, ×4.4.
FIG. 55. *Fuscoboletinus aeruginascens* + *Larix occidentalis* ectomycorrhizae, ×2.6.
FIG. 56. Questionable mycorrhizal colonizations of *Pinus ponderosa* roots by *Suillus grevillei*, ×6.3.
FIG. 57. Questionable mycorrhizal colonizations of *Pinus contorta* by *Suillus grevillei*, ×4.4.
FIG. 58. *Truncocolumella citrina* + *Larix occidentalis* ectomycorrhizae, ×3.2.
FIG. 59. *Zelleromyces gilkeyae* + *Pseudotsuga menziesii* ectomycorrhizae, ×3.8.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1930). Neither typical mantles nor Hartig nets developed in our experiments, and many colonized short roots appeared unhealthy. In Grand's (1968) pure culture syntheses, *S. grevillei* did not form ectomycorrhizae with lodgepole and ponderosa pines, although he, too, observed considerable forking of feeder roots. Similarly, Melin (1922) found *S. grevellei* to produce mantles on *Pinus sylvestris* as well as *Picea abies*; but no true ectomycorrhizae formed.

*Suillus lakei* (Murr.) Smith & Thiers

*S. lakei* formed well-developed ectomycorrhizae of classical anatomy on all hosts except ponderosa pine. Ectomycorrhizae were white to pale brown with a noticeable pink tinge; surfaces varied from loosely tomentose to appressed. Douglas-fir and western hemlock ectomycorrhizae became mostly pinnate or variously branched, 2–10 mm long. Western larch were similar, but longer lateral roots were often colonized and the variously branched ectomycorrhizae measured as long as 20 mm (Fig. 52). Sitka spruce remained simple, 1–7 mm long. Abundant lodgepole and western white pine short roots were colonized and developed into swollen, compact coralloid structures, 4 mm long. Fewer ponderosa pine roots were colonized; and although dichotomously branched, they were less swollen than lodgepole and sparsely mantled.

Cross sections showed well-developed mantles and Hartig nets on all hosts except ponderosa pine (Figs. 61–63). Mantles were mostly tightly interwoven with loose peripheral hyphae and varied in thickness from 10–30 $\mu$m on Douglas-fir to 25–50 $\mu$m on western white pine. Hartig nets were prominent and uniformly penetrated two to three cortical cell layers. A tannin layer was noted in Sitka spruce sections. On ponderosa pine, the mantle was loosely appressed; and although a full Hartig net developed, it widely separated the cells and caused some disruption. Numerous cortical cells stained deeply with safranin and had an overall unhealthy appearance.

*S. lakei* fruits commonly throughout the Pacific Northwest but only in association with Douglas-fir; it has also been reported in exotic plantations of Douglas-fir around the world (Mikola 1970). It was thus unexpected that this particular isolate was able to form such well-developed ectomycorrhizae with nearly all the other conifer hosts.

*Tricholoma flavovirens* (Pers. ex Fr.) Lund.

*T. flavovirens* formed ectomycorrhizae with all hosts. Ectomycorrhizae were white with a distinct pinkish cast; mantle surfaces were mostly appressed with occasional extending wefts of mycelium. Although all ectomycorrhizae were well

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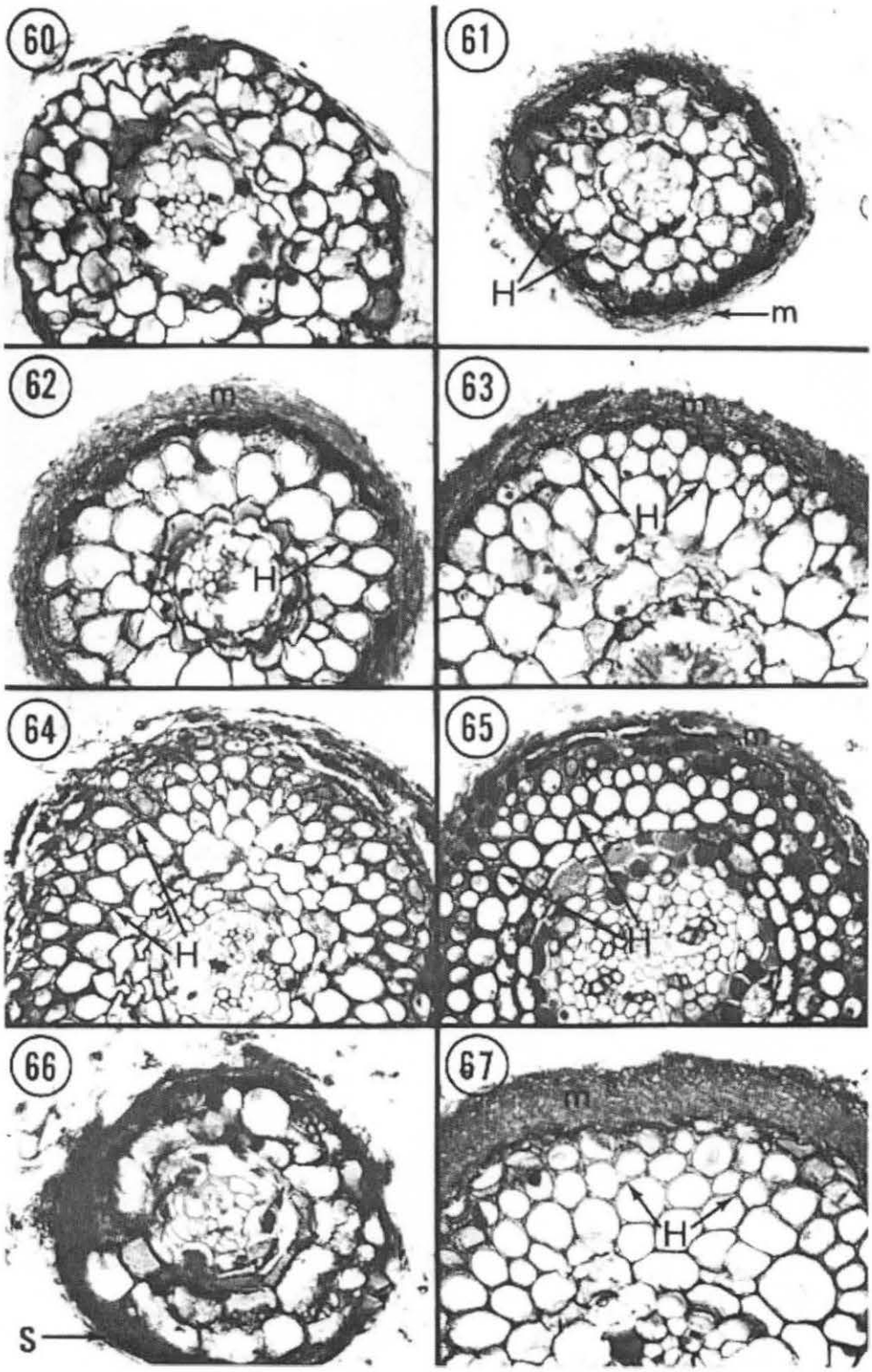
FIGURES 60–67. Cross sections of synthesized ectomycorrhizae and colonized feeder roots. All figures ×170 (m, mantle; H, Hartig net; S, safranin stained cells).

FIG. 60. *Pinus ponderosa* colonized only on the surface by *Suillus grevillei*. Hartig net is lacking.
FIG. 61. *Suillus lakei* + *Picea sitchensis* ectomycorrhiza.
FIG. 62. *Suillus lakei* + *Tsuga heterophylla* ectomycorrhiza.
FIG. 63. *Suillus lakei* + *Pinus monticola* ectomycorrhiza.
FIG. 64. *Truncocolumella citrina* + *Larix occidentalis* ectomycorrhiza.
FIG. 65. *Truncocolumella citrina* + *Pseudotsuga menziesii* ectomycorrhiza.
FIG. 66. *Pinus monticola* colonized by *Truncocolumella citrina*. Note the disruption of the cortex and safranin staining of outer cortical cells.
FIG. 67. *Zelleromyces gilkeyae* + *Pseudotsuga menziesii* ectomycorrhiza.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colonized and colored by the mycelium, they lacked a distinct swollen appearance. Lodgepole and ponderosa pine varied from simple to bifurcate to compound-bifurcate, 1–6 mm long. For all other hosts, ectomycorrhizae remained simple cylindric structures 1–8 mm long.

Cross sections showed mantles to be mostly tightly appressed with occasional loose outer hyphal strands and varying in thickness from 15–30 $\mu$m on lodgepole pine up to 50 $\mu$m on Douglas-fir. Hartig nets uniformly penetrated two to four cortical cell layers. Tannin layers were also common.

We have always collected *T. flavovirens* beneath pines in Oregon, and various pine species are the most common probable hosts listed by Trappe (1962). Norkrans (1949), however, synthesized ectomycorrhizae between *T. flavovirens* and *Betula alba* Roth as well as *P. sylvestris*. Kalmar (1950) also lists *Abies alba* Mill. as a probable host. In the present study neither ectomycorrhizal abundance nor anatomy indicated preference towards a *Pinus* association.

*Truncocolumella citrina* Zeller

*T. citrina* formed classical and abundant ectomycorrhizae with Douglas-fir and western larch. Mycorrhizae were initially pale brown, turning with age to a golden brown with darker brown underlying mycelium. Surfaces were irregularly tomentose with extending wefts of hyphae and occasional appressed to emergent dark brown hyphal strands. Ectomycorrhizae were 1–9 mm long, mostly cylindric on Douglas-fir but becoming irregularly pinnate to variously branched on western larch (Fig. 58). Cross sections showed tightly interwoven mantles 20–50 $\mu$m thick. Well-developed Hartig nets uniformly penetrated two to three cortical cell layers deep (Figs. 64 and 65).

It was difficult to decide whether or not *T. citrina* formed true ectomycorrhizae with any *Pinus* spp. Numerous pine short roots were dichotomously branched and mantled with patchy, loose appearing mycelium. Cross sections, however, showed only fragmentary mantles, absent to 8 $\mu$m thick, and intense safranin staining of cortical cells that gave an overall unhealthy appearance to the tissue. Some intercellular growth was evident but irregular in penetration (Fig. 66).

*T. citrina* is a hypogeous basidiomycete related to *Rhizopogon* spp. and specifically associated with Douglas-fir (Trappe, unpublished data). Yet, equivalent ectomycorrhizae formed on western larch to further indicate the ability of these two hosts to share highly specialized symbionts. *T. citrina* appeared incompatible with *Pinus* spp.

*Zelleromyces gilkeyae* Sing. and Smith

*Z. gilkeyae* formed ectomycorrhizae only with Douglas-fir and western hemlock. It penetrated the peat-vermiculite substrate slowly thus colonizing few feeder roots. Ectomycorrhizae were conspicuously swollen, translucent white when young, becoming pale orange brown with age; mantle surfaces were smooth. In overall appearance these ectomycorrhizae highly resembled those of *Lactarius deliciosus*. Western hemlock ectomycorrhizae remained short and nub-like, 1.5–3 mm long. Mature Douglas-fir mycorrhizae became irregularly pinnate to variously branching, 1.5–12 mm long (Fig. 59).

Cross sections showed tightly interwoven mantles of fine hyphae appressed at the surface. Thickness varied from 15–40 $\mu$m on western hemlock to 35–50 $\mu$m on Douglas-fir. Hartig nets uniformly penetrated two to four cortical cell layers (Fig. 67).

This is the first report of ectomycorrhiza formation by a *Zelleromyces* sp. *Z. gilkeyae* is predominantly collected beneath Douglas-fir but collections in mixed Douglas-fir-western hemlock and occasionally with western hemlock alone have

been noted (Trappe, unpublished data). Our results confirm the specific ectomycorrhizal association with these two hosts with best development occurring on Douglas-fir.

*Zelleromyces* is a genus of hypogeous Basidiomycetes in the Russulaceae (Shaffer 1968). Although sporocarps are not agaricoid, their production of latex and amyloid spore ornamentation indicate phylogenetic relationship to *Lactarius* spp. (Singer and Smith 1960) as now further evidenced by the close similarity of ectomycorrhizae formed by *L. deliciosus* and *Z. gilkeyae*.

### Comparison of Patterns of Ectomycorrhizal Host Potential and Specificity

From Table 2 and the previous descriptions, the fungi varied widely in ectomycorrhizal host potential and specificity among the various conifers in a spectrum from generalists to specialists. In general, however, the fungi can be placed into three groups: (1) fungi with wide ectomycorrhizal host potential, low specificity, sporocarps usually associated with diverse hosts in the field, (2) fungi with intermediate host potential yet specific or limited in sporocarp-host associations, and (3) fungi with narrow host potential, only able to form ectomycorrhizae with a specific host species or genus and limited likewise in sporocarp association.

Readily apparent at one end of the spectrum were the numerous fungi of group 1 which formed ectomycorrhizae, usually abundantly, with all of the conifers including the oft studied species with known wide host ranges: *Amanita muscaria, Boletus edulis, Cenococcum geophilum, Laccaria laccata, Lactarius deliciosus, Paxillus involutus,* and *Pisolithus tinctorius*. Four other species, with poorly known host ranges also displayed wide ectomycorrhizal host potential. Two are Gastromycetes, *Astraeus pteridis* and *Scleroderma hypogaeum,* one a hypogeous Basidiomycete, *Melanogaster intermedius,* and the fourth *Tricholoma flavovirens*. Nearly all ectomycorrhizae formed by these fungi were well developed, easily discernable as well as constant in external appearance, and without superficial or questionable characteristics. Except for *Paxillus involutus,* Hartig nets developed regularly in all cases.

At the opposite end of the spectrum are the more specialized fungi of group 3, which displayed narrow ectomycorrhizal host potential. *Alpova diplophloeus* was the most specialized: it did not form ectomycorrhizae with any of the conifers, although it produced well-developed ectomycorrhizae with five *Alnus* species under identical cultural conditions (Molina 1979, 1981) without the disruption noted on colonized conifer roots. Two fungi, *Cortinarius pistorius* and *Rhizopogon cokeri,* were limited to pines. *C. pistorius* was only weakly mycorrhizal in this regard, however, and *R. cokeri* is also known to form ectomycorrhizae with *Tsuga mertensiana* in the field. *Gastroboletus subalpinus* and *R. occidentalis* showed a similar preference for pines, but *G. subalpinus* also formed a well-developed ectomycorrhiza with Douglas-fir while *R. occidentalis* produced a few weakly developed ectomycorrhizae with Sitka spruce. Two fungi formed ectomycorrhizae with only two hosts: *Zelleromyces gilkeyae* was restricted to Douglas-fir and western hemlock, and fruits in association with both. *Truncocolumella citrina,* which has been reported fruiting only under Douglas-fir, also formed ectomycorrhizae with western larch.

The strongly host-specific fungi in the genera *Suillus* and *Rhizopogon* were predominant in intermediate group 2 and provided the most interesting and variable patterns of ectomycorrhizal host potential. Each fungus formed anatomically classical ectomycorrhizae with at least one unexpected host (i.e., no known sporocarp association) and often with two or more. *Suillus* spp. generally displayed wider ectomycorrhizal host potential than *Rhizopogon* spp. For example, *Suillus*

*lakei*, which reportedly fruits only under Douglas-fir, formed ectomycorrhizae with all hosts. *Suillus brevipes*, which reportedly fruits under pines and *Tsuga mertensiana*, did nearly the same. On the other hand, *Rhizopogon vinicolor*, *R. occidentalis*, and *S. grevillei* formed ectomycorrhizae with only one or two unexpected hosts.

The fungi of group 2 often showed greater specialization and preference towards hosts under which they fruit, e.g., "pine" fungi usually developed the best ectomycorrhizae on pines. Even though regularly developed Hartig nets satisfied the criterion for judging the ectomycorrhizal status of the unexpected unions, the proportion of short roots colonized and degree of mycorrhizal branching was often less than on the sporocarp-associated host. In many unexpected associations, the short roots were colonized only at their base or the ectomycorrhizae remained short and simple with a poorly developed mantle. Hartig nets at times separated the cortical cells widely and appeared to disrupt the cortical tissue. Safranin staining of cortical cells was more common on unexpected hosts than sporocarp-associated hosts. These differences in ectomycorrhizae formation may relate to the degree of specialization which has evolved in these associations. Growth substances produced by both symbionts influence the development of ectomycorrhizae (Slankis 1973). It appears that many highly specialized, host-specific fungi produce hormones or other metabolites which stimulate only the specific host(s). In return, the fungi may receive particular growth substances needed to complete their life cycle only from specific hosts, accounting for the specific host association of sporocarps in the field.

Superior development of ectomycorrhizae on the specific host, however, was not true for all the fungi that fruit only in association with that host. For example, *Suillus lakei* formed ectomycorrhizae equally well with both western hemlock and larch as on Douglas-fir. Similarly, *Leccinum manzanitae*, reportedly fruiting strictly with *Arbutus* and *Arctostaphylos*, formed classic ectomycorrhizae of equal status on five conifers as on either ericaceous host. In one unexpected synthesis, *Truncocolumella citrina* + western larch ectomycorrhizae developed better than on the "specific" host, Douglas-fir. Thus, even specialized fungi appear to have greater complexity in ectomycorrhizal host potential and specificity than previously conceived.

Fewer obvious patterns appeared in comparing the ectomycorrhiza-forming abilities of the various hosts. Their ability to form ectomycorrhizae with the broad host-ranging fungi did not differ. The three pine species appeared nearly equal in ability to form ectomycorrhizae among both the natural pine-specific fungi and the broad host-ranging fungi. Neither our data, Suggs and Grand (1972), nor Trappe's (1962) listing of pine-specific fungi indicate strong infrageneric differences towards potential fungus associates within *Pinus*. The same is true for *Alnus* (Molina 1981) and *Eucalyptus* (Malajczuk and others 1982).

Douglas-fir and western larch show similarity in ability to form ectomycorrhizae among the various fungi. Most fungus symbionts that fruit only with the one formed ectomycorrhizae with the other. Because western hemlock and Douglas-fir often associate in mixed stands throughout the Pacific Northwest, we suspected western hemlock might also share Douglas-fir-specific symbionts. This proved not the case. *Suillus lakei* developed profuse ectomycorrhizae on western hemlock but did likewise on other hosts. *Rhizopogon vinicolor* only weakly colonized western hemlock as has been seen with other Douglas-fir-specific *Rhizopogon* species as well (Molina 1980b). The remaining Douglas-fir associate, *Truncocolumella citrina*, failed to initiate ectomycorrhizae with western hemlock.

No special patterns of ectomycorrhiza formation were noted for Sitka spruce. Ectomycorrhizae formation was always best with the broad host-ranging fungi. Although fairly well-developed ectomycorrhizae did form with some of the spo-

rocarp-host specific fungi, e.g., *Fuscoboletinus aeruginascens* and *Suillus lakei*, more often than not the associations were poorly developed and of questionable ectomycorrhizal status. Unlike *Pinus*, *Larix*, and *Pseudotsuga*, for which many genus-specific fungi are known, we are unaware of *Picea*-specific ectomycorrhizal fungi. More intense collecting in *Picea* habitats with this purpose in mind, however, may yield such specific fungi.

### Indications of Incompatibility

The consistently well-developed ectomycorrhizae produced on all hosts by the broad host-ranging (group 1) fungi certainly indicate little host incompatibility. These unspecialized fungi may share a compatibility or recognition factor common to many or all ectomycorrhizal hosts. These fungi also induced considerable branching on ectomycorrhizae of most hosts. In contrast, the more specialized fungi of group 2 often only induced morphological changes on their sporocarp-specific hosts. Slankis (1973) has emphasized the role of hormones in ectomycorrhiza formation and development. How these groups of fungi differ in their hormonal interactions with various hosts and whether these interactions play a role in determining ectomycorrhizal compatibility and specificity are areas for future research.

For many of the unsuccessful syntheses with fungi such as *Cortinarius pistorius*, *Gastroboletus subalpinus*, and *Zelleromyces gilkeyae*, anatomical indications of incompatibility with host tissues were not obvious. Short roots often were not colonized, remained undifferentiated, and bore root hairs. Colonization of unassociated hosts by many of the host-specific fungi, however, often elicited host responses indicating incompatibilities, e.g., between the three pine species inoculated with Douglas-fir-specific fungi and vice versa. Disruption of the cortex by the invading fungus and lignification as indicated by intense safranin staining of cortical cells were the most common indicators. Thin and fragmentary mantles with underlying cortical cells exhibiting thickened, lignified, collapsed walls were also common. Hyphal penetration between these cells occurred rarely and, when present, often disrupted cortical tissue.

Lignification entails deposit of polyphenolic compounds into the secondary cell wall and is regarded as an active defense against pathogenic invasion (Hijwegen 1963, Friend 1979). Such a reaction to colonization by incompatible mycorrhizal fungi seems also to be active resistance to fungal penetration or, because many of these cells collapsed, a hypersensitive response. Many such fungi penetrated this tissue little or not at all. Similar reactions have been described among incompatible fungi and hosts in previous studies (Molina 1981, Malajczuk and others 1982). It should be noted, however, that a few well-developed ectomycorrhizae formed by fungi, such as *Paxillus involutus*, produced similar host reactions, yet the fungus formed a Hartig net, indicating a differential tolerance to such host responses on the part of the fungi. Other roles phenolics may play in determining ectomycorrhizal compatibility and specificity have been discussed by Molina (1981).

## SUMMARY AND CONCLUSIONS

Host specificity in ectomycorrhizal associations has received scant attention in the past; most references are limited to citing field observations of specific sporocarp-host associations. This study has emphasized that such a simplified view of ectomycorrhizal specificity is incomplete. Results from this study and others (Grand 1968; Linnemann 1971; Molina 1979, 1981; Molina and Trappe 1982; Malajczuk and others 1982) conclusively demonstrate Trappe's (1962) earlier suggestion that fungi specific to a particular host species or genus in sporocarp

association are not necessarily limited likewise in their ability to form ectomycorrhizae with other hosts. We have termed this ability to form ectomycorrhizae, at least under these restricted cultural conditions, as ectomycorrhizal host potential. How this ectomycorrhizal host potential operates in natural systems remains to be determined.

A wide variety of ectomycorrhizal host potentials were displayed by the fungi in this study. Many displayed strikingly wide ectomycorrhizal host potential as exemplified by their ability to form well-developed ectomycorrhizae with all hosts and, in most cases, by their fruiting in association with many host species in the field. These fungi may share a common or similar compatibility or recognition factor which allows them to enter into ectomycorrhizal association with diverse hosts.

The greatest variation in ectomycorrhizal host potential was seen among fungi that fruit only under particular hosts. Their ectomycorrhizal host potential ranged from a total inability to form ectomycorrhizae with any conifer host, e.g., the *Alnus*-specific symbiont *Alpova diplophloeus*, to forming ectomycorrhizae with all or most hosts tested. More often than not, however, ectomycorrhizae development was best on the sporocarp-associated hosts. This was especially evident in the degree of feeder roots colonized and in stimulation of branching and elongation. Thus, although their ectomycorrhizal host potential may not be as limited as their sporocarp production, they still express a high degree of preference towards their sporocarp-specific host.

Further specialization of the sporocarp-host specific fungi was seen in their apparent incompatibility with other hosts. Disruption of the cortex by the colonizing fungus was often noted in incompatible host-fungus confrontations. Cortical cells adjacent to the mantling mycelium became lignified and were often in various stages of collapse. This host reaction suggests a type of phenolic defense mechanism as displayed in many plant-parasite interactions. Possible roles that phenolics may play in determining ectomycorrhizal compatibility and specificity and other mechanisms that may similarly be involved in these expressions have been discussed by Molina (1981).

In conclusion, a complex variety of potentials, specificities, and compatibilities has been demonstrated among ectomycorrhizal symbionts. Research can now focus on the specific mechanisms involved. If phenolics play a role, we must characterize them and use histochemical techniques to find their cellular origin. Differences in enzyme precursors needed to produce such chemicals, e.g., laccase, peroxidase, phenoloxidase, must be compared between compatible and incompatible host-fungus combinations. The exchange of hormones and other growth metabolites between symbionts must be further characterized and compared in this regard. Data on net host response, e.g., enhanced mineral uptake and top and root growth, to mycorrhizal colonization will be important in interpreting the symbiotic status and efficiency of given fungus-host combinations. Finally, these interactions must be looked at in relation to the complex of natural environmental and microbiological factors which are known to affect ectomycorrhiza formation.

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